CHARACTERISTICS OF LEAF RUST FUNGI OF TEMPERATE CEREALS AND THEIR HOST RELATIONSHIPS

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DECLARATION

This is to declare that this thesis has been composed by myself and has not been accepted in any previous application for a degree. The work of which it is a record has been done by myself and all sources of information have specifically been acknowledged by means of reference.

Stephan Helfer

June 1986

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SUMMARY

This thesis describes work carried out mainly on the uredinial stage of the leaf rust fungi of temperate cereals, comprising the brown rusts of barley, rye and wheat as well as oat crown rust and the wheat and barley yellow rusts.

The taxonomic position of these pathogens was investigated in colony growth studies carried out under controlled conditions, with an emphasis on characters which were independant of cereal host factors. A clear morphological distinction could be made between oat crown rust, wheat brown rust, barley brown rust, rye brown rust and the yellow rusts of wheat and barley. However, no distinction apart from the host range could be made between wheat and barley yellow rust.

In culture experiments the isolates were tested for their ability to grow on detached leaves of their host plants, and some of the isolates were used in axenic culture studies. All the isolates showed identical virulence patterns on detached leaves as on whole host plants. None of the isolates tested could be maintained in axenic culture.

Cytogenetical characteristics were studied in some of the rust isolates. The dikaryotic binucleate condition prevailed in all isolates up to the stage of stomatal penetration. Attempts to obtain hybridisation between different races of wheat yellow rust failed.

Quantitative aspects of colony growth and development were investigated in a series of studies in controlled conditions. Differences in response to inoculum density were observed in experiments with barley brown rust and yellow rust and were related to

the different patterns of epidemic development of these two species. The colony growth patterns of the cereal leaf rusts in host and non-host relationships showed strong host preference. However, unexpected colony development occurred in some of the pathogen / non-host combinations.

CONTENTS

CHAPTER 1

General introduction

Among the cereal pathogens the rust fungi, comprising the order Uredinales, present the most interesting and economically important group. Biologically they are remarkable through their complicated life cycle, their obligate biotrophic existence and their host specificity.

According to Savile (1984) there are 10 distinct rust species attacking cereals, six of which are specialised on the temperate genera of oats, barley, wheat and rye. These six species can be classified into two groups, the stem rust diseases caused by Puccinia graminis and the leaf rust fungi, comprising oat crown rust (Puccinia coronata), barley brown rust (Puccinia hordei), rye brown rust (Puccinia recondita), wheat and barley yellow rust (Puccinia striiformis), and wheat brown rust (Puccinia triticina). With the exception of P. striiformis, the leaf rusts have a complicated life cycle involving an alternation between two systematically very different host plants together with a regular change between a monokaryotic haploid and a dikaryotic status with five different spore stages (Gaeumann, 1949; Fig. 1.1). In the spring teliospores, which overwintered on straw, germinate to form the basidiospores (designated stage "IV"). When these make contact with young leaves of the alternate host plant they germinate and form a haploid mycelium which leads to the formation of pycniospores or spermatia (spore generation "0"). After dikaryotisation (plasmogamy), which involves the pycniospores (Craigie, 1927), specialised receptive organs of the fungus and often insect vectors

(Buller, 1950; Littlefield & Heath, 1979), later in the spring, another spore generation, the aeciospores ("I"), is formed on the alternate hosts. These spores are dikaryotic and cannot infect the alternate hosts any more. They are normally air-borne and lead to the development of a dikaryotic mycelium on the main host. The alternate and main hosts and the different stages in the life cycles of the cereal leaf rusts are presented in Fig. 1.1.

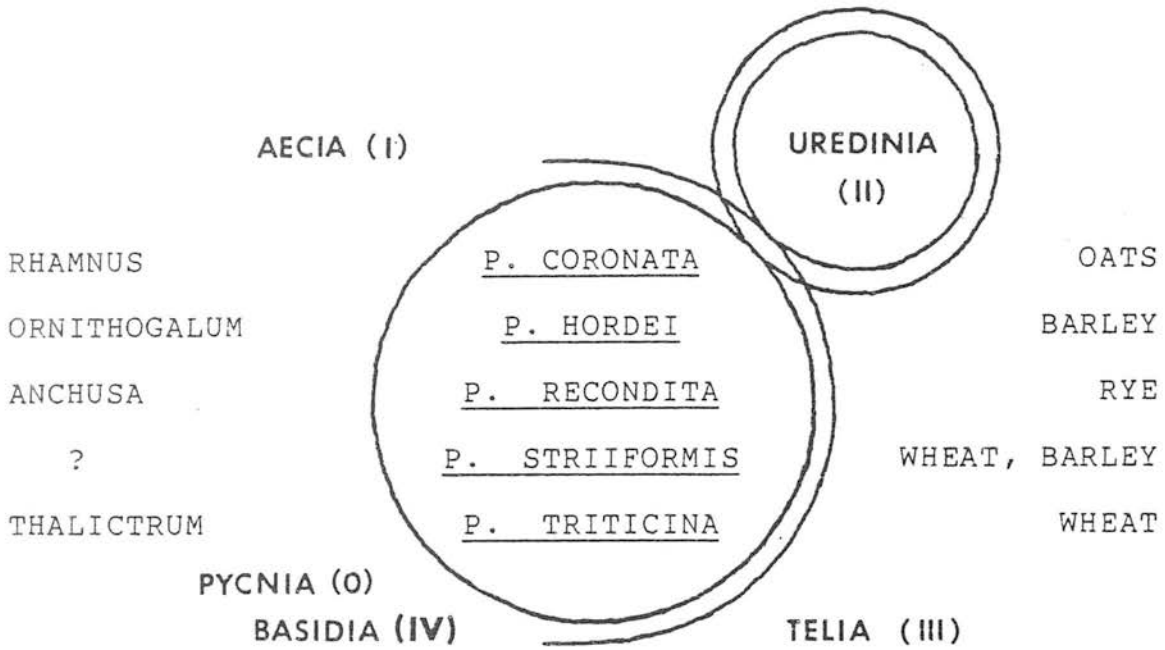


Fig. 1.1 The life cycle of the cereal leaf rusts on main and alternate hosts.

During the summer the dikaryotic mycelium leads to the formation of urediniospores (spore generation "II"), which are the main means of

propagation for the fungus, as this spore stage can be repeated many times in favourable conditions. Towards the end of the summer the dikaryotic mycelium in the main host produces the characteristically two-celled, thick walled teliospores (generation "III"), the overwintering spores for the next season. During the late autumn the two nuclei in each teliospore cell fuse to form a diploid nucleus. With the germination of the teliospore in the next spring, the nuclei undergo reduction division and four monokaryotic haploid basidiospores are formed. And here begins the cycle anew. An alternate host, pycnia or aecia, have not yet been found for P. striiformis.

The economically most significant and also the most widespread spore stage of the cereal rusts is the urediniospore stage. It is important that a clear distinction between different rust species can be made: while knowledge of the host plant on which urediniospores have been found is not always sufficient for a definite determination, the five leaf rust species differ slightly in their morphology and microscopic examination can reveal their identity. In Chapter 2 taxonomic aspects of the leaf rusts in their urediniospore stage are discussed.

A biologically interesting feature is the obligate biotrophy of rusts. This means that these fungi, similar to the downy mildews and the powdery mildews, can grow only in the presence of living host tissue. Only relatively recently (Williams et al., 1967) has it been possible to grow members of this order in axenic culture, and many rusts still resist the attempts of scientists to grow them in vitro. Growing parasites in the absence of their hosts has a number of interesting implications (Williams, 1984): firstly in studying

physiological, genetical and morphological aspects of the parasite alone and secondly in carrying out experiments concerning these aspects. Questions on the culture of rusts, including experiments aimed at the establishment of axenic culture, are considered in Chapter 3.

The cereal rusts are highly specialised on their hosts. Not only is their development restricted to a relatively small number of host species, both as main and alternate hosts (Wahl et al., 1984), but they also show specialisation on distinct host cultivars existing as a great number of physiologic races. These are morphologically identical forms of a rust species which show differences in their virulence patterns on different host cultivars. Physiologic races were first discovered in the wheat stem rust fungus P. graminis (Stakman & Piemeisel, 1917; Hoerner, 1919) and later in other cereal rusts (Mains, 1926; Mains & Jackson, 1926; Allison & Isenbeck, 1930; Gassner & Straib, 1932a). Flor (1942) proposed a model for the genetical background of physiologic race differentiation, known as the gene for gene hypothesis. It says that host genes for resistance to a disease are met and overcome by specific virulence genes in the parasite. This hypothesis has been found true in many host-pathogen interactions (Flor, 1971) and is recognised by the plant breeders who may aim to combine specific resistances. Yet, the rust parasites have also means of recombination in their virulence structure, and it has been reported (Bayles & Thomas, 1984) that new resistance combinations in cereal varieties have been overcome by the rust in relatively short time. Mechanisms of recombination phenomena in the parasites are biologically interesting and economically relevant. Some aspects of these phenomena are discussed in Chapter 4.

Although many studies have been carried out on the physiological factors affecting cereal rust fungi and their host relationships, there is still a need to develop further our knowledge on these aspects. Chapter 5 is concerned with physiological studies relating to spore germination, the effect of inoculum density on subsequent spore production and the development of rust fungi on hosts and non - hosts.

Apart from their biological interest the cereal rust fungi are very important economically as the causal organisms of serious diseases of the world's cereal crops. One of the most severe cereal diseases until this century was black stem rust caused by P. graminis. It was already known to the ancient Romans (Lehmann et al., 1937; Zadoks, 1985), and some of the plant diseases mentioned in the Bible can be interpreted at least partly as this rust (e.g. Deuteronomy 28,22). Even before the discovery of the life cycle of P. graminis by de Bary in 1866 (Schafer et al., 1984), the use of sanitary measures with the eradication of its alternate host, the barberry, was introduced (Lehmann et al., 1937) and, together with the extensive use of genetical resistance, this rust has become second in importance on a world scale behind the wheat brown rust caused by P. triticina (Chester, 1946; Samborski, 1984; Saari & Prescott, 1984). The yield losses caused by rusts are substantial (Batts & Elliot, 1952; Doling & Doodson, 1968; King & Polley, 1976; Singleton et al., 1982; Shteinberg et al., 1984) and therefore great effort is being invested into their control. This can be achieved by breeding for resistance, improvement of chemical control measures using fungicides and crop husbandry measures. To ensure maximum success in disease control a good knowledge of the biology of the parasites involved is necessary. The present studies

are aimed to provide further information on the taxonomic position of the cereal leaf rusts, their culture, their cytogenetical properties, their physiological requirements and growth and development characteristics.

CHAPTER 2

Taxonomic studies

Introduction:

The systematic classification of fungi at the species level is based mainly on morphological data, as physiological properties and breeding behaviour cannot be preserved in herbarium specimens. The biotrophic life style of the rust fungi has led to two lines of thought, one emphasising the host range at the species level together with morphological characters, the other relying only on the morphological features irrespective of the host on which the fungus occurred. The species concept with both approaches is artificial and it is questionable how far they can reflect phylogenetical relationships.

Since the beginning of the century until the 1960s, the aim of most researchers was to find taxonomic units in the rust fungi which could be described by their host range as well as their morphology. Thus many new rust species were recognised as distinct taxa, although their morphological features were in some cases indistiguishable and only quantitative variation was evidenced. A culmination of these studies was the book by Ernst Gaeumann (1959), "Die Rostpilze Mitteleuropas". In this publication around 2000 rust species were described for Central Europe. Joining them into morphologically and physiologically similar groups ("Formenkreise"), he achieved a system of clear differentiation, once the host was identified. This system was based on an assumption of strict host specialisation. Where very similar rusts appear on the same host, as in the case of wheat and rye brown rusts on

rye, differentiation is, however, difficult. In a more recent book Wilson and Henderson (1966) approached the British rust fungi from a purely morphological point of view, and described only about 250 species with a number of varieties and formae speciales for the British Isles. The present investigation was aimed to examine further from their uredinial stage the systematic position of the cereal leaf rusts listed below:

Puccinia triticina Eriksson, wheat brown rust (WBR)

P. striiformis Westendorp, wheat and barley yellow rust (WYR, BYR)

P. hordei Otth, barley brown rust (BBR)

P. recondita Roberge ex. Desmellier, rye brown rust (RBR)

P. coronata Corda, oat crown rust (OCR)

Materials and methods:

Six isolates of P. triticina (WBR), 10 isolates of P. striiformis (WYR) and one of P. striiformis (BYR), four isolates of P. hordei (BBR) and one each of P. recondita (RBR) and P. coronata (OCR) were artificially inoculated onto their respective hosts using a set of universally susceptible cereal cultivars. Host plants were grown at 14 $\pm$ 2 °C in compost (Levington's potting compost) in 12 cm pots for whole plant experiments or in trays for detached leaf experiments. Host seedling leaves were used when they had fully expanded (10 to 14 days after sowing). All plants were grown in spore free cabinets and whole plants were kept under polythene domes with a slight positive air pressure to avoid contamination during the period of the experiment. The growth of true leaves was removed at 2-day intervals to avoid excessive foliar growth in the domes. Spores of the rust isolates were brushed onto intact plants with cotton buds or dusted onto detached

Table 2.1
Morphological features of cereal leaf rusts.

Structural property	Rust isolates *) OCR	BBR	RBR	YR **)	WBR
Sorus size µm	130 X 790	180 X 401	359 X 1121	158 X 343	200 X 780
S.E.	56 540	60 185	124 605	49 98	120 527
Paraphyses	rare	rare	present	rare	present
Colour of dry spores	light brown	medium brown	dark brown	orange	dark brown
Spore size µm	16 X 22	17 X 23	19 X 23	17 X 22	18 X 23
S.E.	1.3 1.8	1.7 1.9	1.9 2.4	2.0 1.9	1.5 2.0
Echinulation width µm	1.44	1.77	1.83	1.45	1.55
S.E.	0.05	0.10	0.24	0.32	0.24
Number of germ pores	9 - 12	6 - 8	6 - 8	7 - 9(-14)	(6-) 7 - 9

*) O = Oats CR = Crown Rust **) Wheat and Barley
B = Barley BR = Brown Rust Isolates
R = Rye YR = Yellow Rust
W = Wheat

Table 2.1 continued

Structural property	Rust isolates *) OCR	BBR	RBR	YR **)	WBR
Germ tubes	unbranched	branched	branched	unbranched	unbranched
Appressorium size µm	16 X 20	15 X 29	15 X 33	NONE	15 X 27
S.E.	2.4 3.0	2.8 5.7	3.2 1.8	-	2.2 2.3
Sub stomatal vesicle size µm	8 X 45	9 X 41	9 X 58	18 X 27	15 X 21
S.E.	2 5	1 6	2 7	1 4	1 2
Hyphae diam. µm	3.15	3.65	4.94	***) 7(12-14)	3.67
S.E.	1.12	1.22	1.18	1.2 (-)	1.18
Haustorial mother cell size µm	3.3 X 11.0	3.6 X 14.7	5.7 X 11.0	7.0 X 8.0	5.7 X 11.7
S.E.	0.1 1.2	0.1 1.0	1.4 2.0	0.6 1.2	1.7 2.2
Mycelium	loose	dense	loose	dense	medium

*) O = Oats, B = Barley, R = Rye, W = Wheat
CR = Crown Rust, BR = Brown Rust, YR = Yellow Rust
**) Wheat and Barley Isolates
***) Runner hyphae

leaves supported on benzimidazole agar. Detached leaves were incubated at 14 ± 1 °C in growth cabinets. Samples from both experiments were taken after various intervals, dried to provide herbarium specimens or prepared for examination using light microscopy (LM), fluorescence microscopy (FM) or scanning electron microscopy (SEM) techniques (Appendix 2.1-2.3). Three main features were examined: the sorus structure and spore characteristics; germination characteristics; characteristics of vegetative structures (hyphae, haustorial mother cells, growth patterns). The sorus size was measured after the sori had stopped growing (19 to 21 days after inoculation). The spore size was determined using vacuum dried spores.

Results:

The results of morphological examinations are summarised in Table 2.1.

Puccinia coronata was distinguished from the other leaf rusts by the light brown colour of its urediniosori (Plate 2.1) and the

Plate 2.1 Urediniosorus of P. coronata on oats; LM; X 80.

Plates 2.2 - 2.11 P. coronata.

Plate 2.2 Peripheral paraphyses and spores in urediniosorus;
FM; calcofluor stain;
length of bar = 50 µm.

Plate 2.3 Urediniospore showing germ pores (GP) and circular ridges surrounding the spines;
SEM;
length of bar = 10 µm.

Plate 2.4 Germinating urediniospore on water agar; unbranched germ tube with disorientated tip (◄);
SEM;
length of bar = 100 µm.

Plate 2.5 Germinating urediniospore on nutrient agar; slightly branching germ tube; note change in germ tube morphology (◄);
LM; live, unstained;
length of bar = 50 µm.

Plate 2.6 Germinating urediniospore on water agar; small branch in germ tube (◄);
LM; live, unstained;
length of bar = 10 µm.

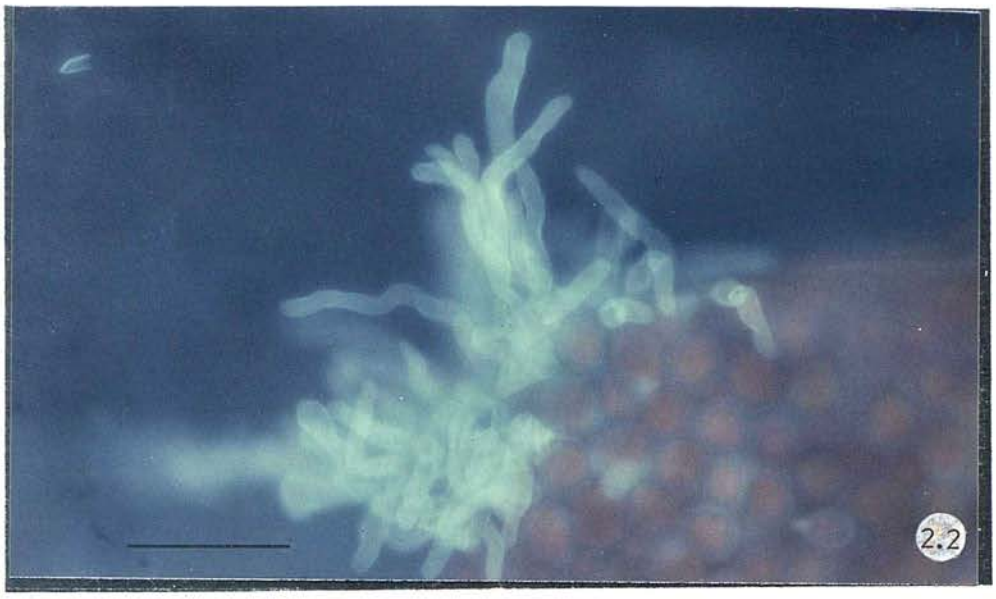
Plates 2.7 & 2.8 Substomatal vesicles with septa (◄) and infection hyphae (IH);
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.9 Haustorial mother cell (HMC); haustorial neck (◄); stomatal guard cells (*) of host plant;
FM; calcofluor stain;
length of bar = 5 µm.

Plates 2.10 & 2.11 Development of urediniosorus:

Plate 2.10 10 days after inoculation;

Plate 2.11 18 days after inoculation; secondary urediniosori erupting around initial sorus;
LM;
X 10.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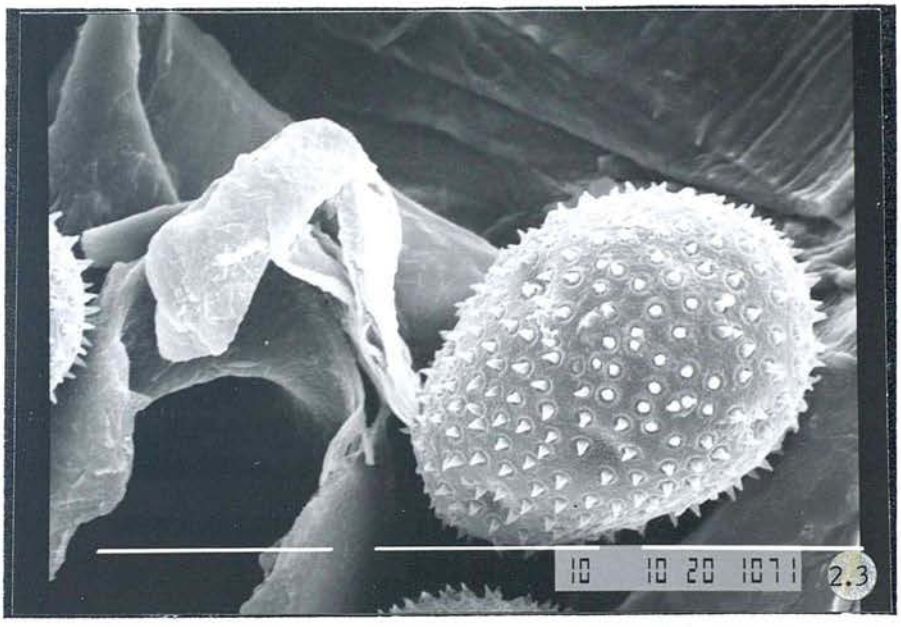
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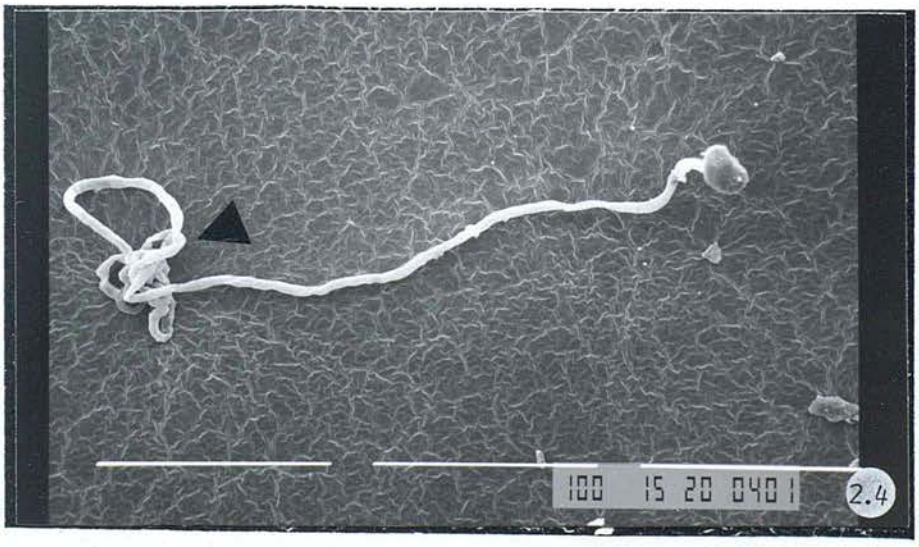
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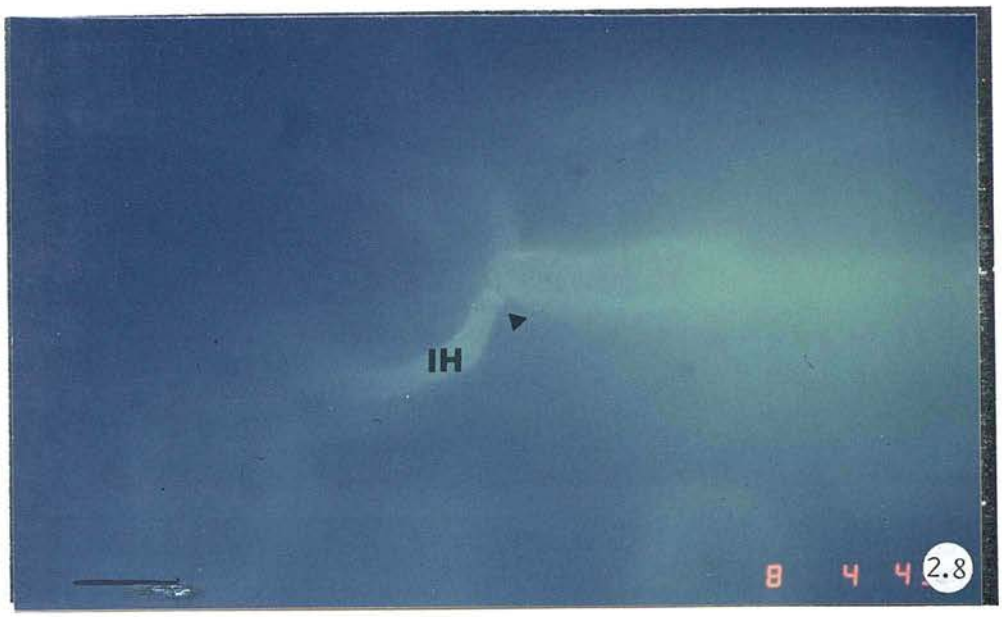
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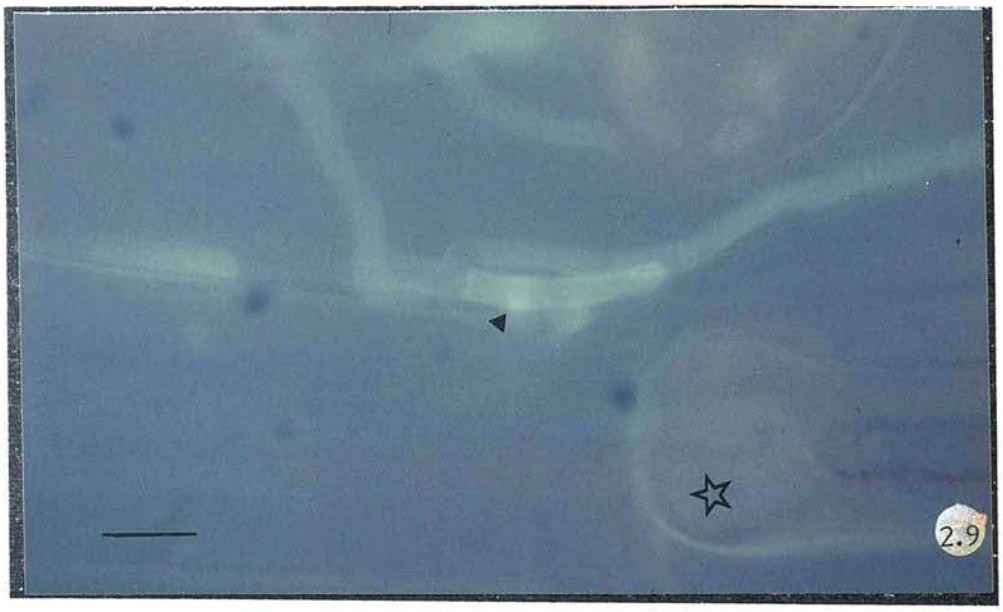
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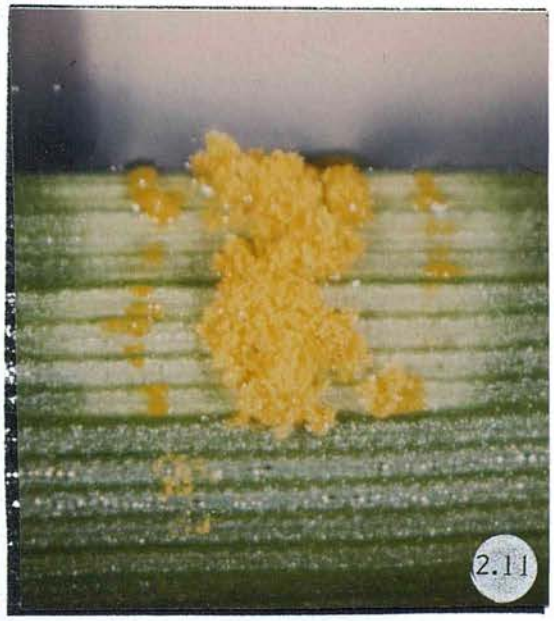
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formation of sori which often occurred in small groups. The sori were found to be relatively narrow but could reach a considerable length. No paraphyses could be observed inside the sori but at their edges hyphae were sometimes extruded (Plate 2.2). The spores were relatively small and pear shaped (Plate 2.3); the echinulation was very distinct and small circular ridges surrounded every spine. The spores each possessed 9-12 germ pores. The germ tubes were mostly unbranched (Plate 2.4) apart from occasional small branches (Plates 2.5 & 2.6). The appressoria were the shortest of those of all the leaf rusts producing appressoria, and the substomatal vesicles were long and narrow (Plates 2.7 & 2.8). Oat crown rust was found to spread relatively rapidly inside host tissues. At frequent intervals long and narrow haustorial mother cells were formed from the the intercellular hyphae (Plate 2.9) which formed a loose mycelium: the hyphae were the narrowest of the rusts examined. Shortly after the eruption of each initial sorus secondary sori were formed closely alongside (Plates 2.10 & 2.11).

Puccinia hordei, the brown or dwarf rust of barley, produced scattered "rust" brown sori (Plate 2.12) of small to medium size. A few

Plate 2.12 Urediniosorus of P. hordei on barley; LM; X 10.

Plates 2.13 - 2.24 P. hordei.

Plate 2.13 Sterile hyphae protruding at sorus edge;
FM; calcofluor stain;
length of bar = 10 µm.

Plates 2.14 & 2.15 Early stages of spore germination; germ pores (◄); germ tube initials ;
SEM;
length of bar = 10 µm.

Plate 2.16 Young and mature urediniospores; note the circular ridges arround the spines;
SEM;
length of bar = 10 µm.

Plate 2.17 Germinating urediniospore on barley leaf; initial secondary germ tube (*); germ tube branches (◄); appressorium (A);
SEM;
length of bar = 100 µm.

Plate 2.18 Appressorium on barley stoma;
SEM;
length of bar = 10 µm.

Plate 2.19 Substomatal vesicle; septa (◄) and infection hyphae (IH);
FM; calcofluor stain;
length of bar = 10 µm.

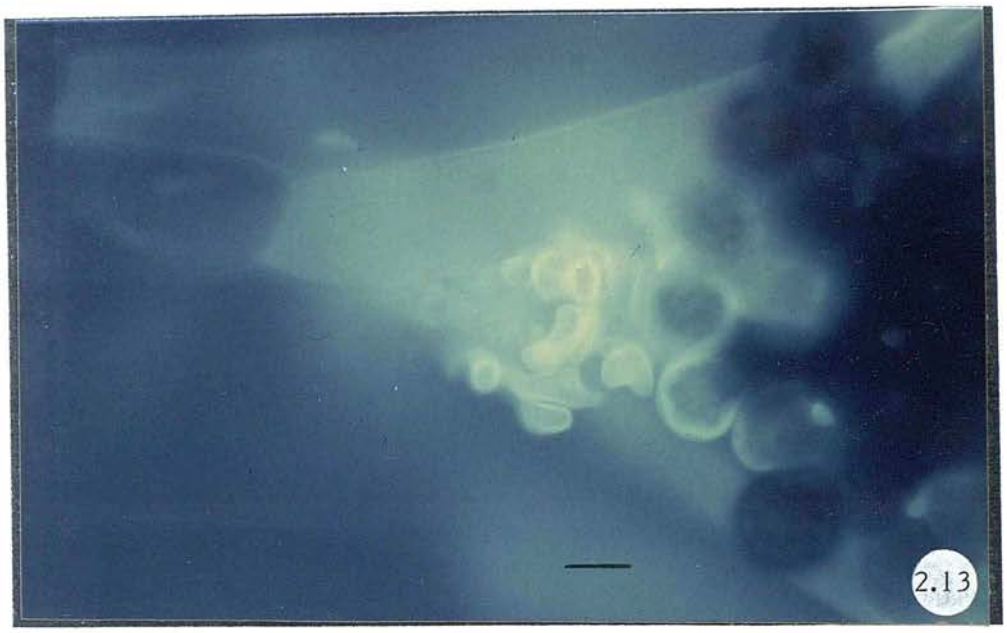
Plate 2.20 Young sorus; note hyphal conglomeration and spore initials (*);
FM; calcofluor stain;
length of bar = 100 µm.

Plate 2.21 Intercellular mycelium near sorus; haustorial mother cell (*);
SEM;
length of bar = 10 µm.

Plate 2.22 Haustorial mother cell at host mesophyll cell;
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.23 Secondary uredinia around initial sorus; note ring of host chlorosis;
LM;
X 80.

Plate 2.24 Green island formation on fully susceptible barley;
LM;
X 6.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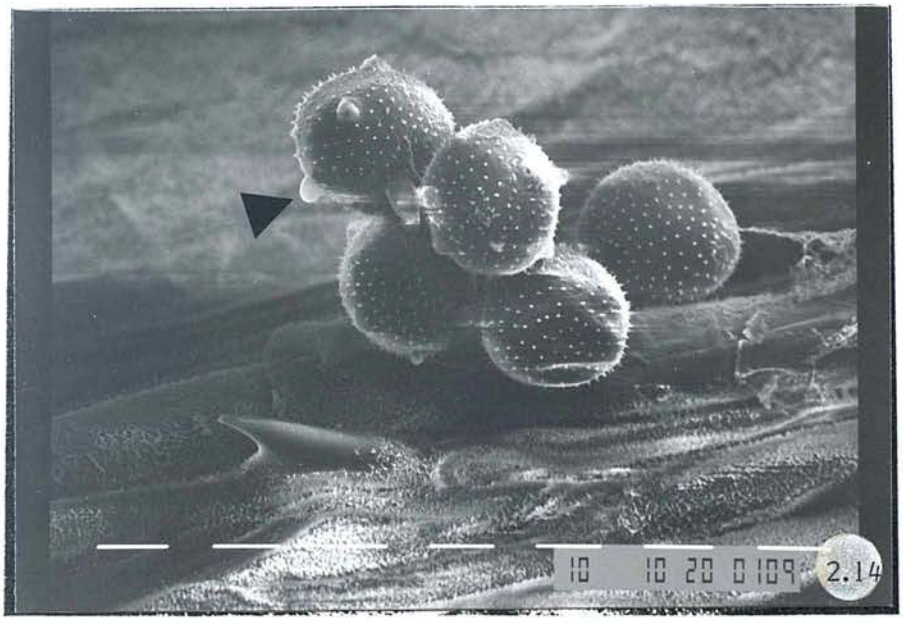
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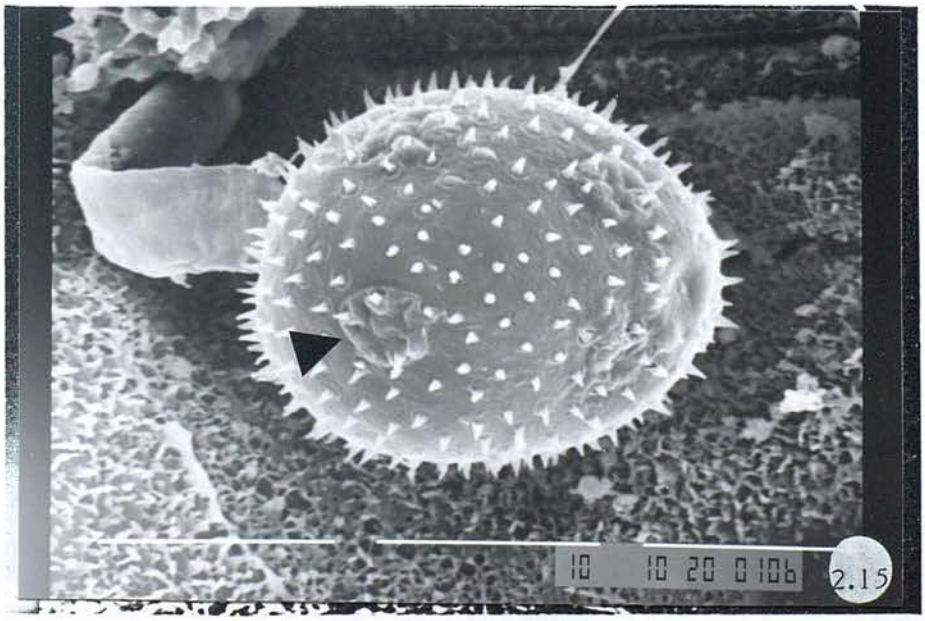
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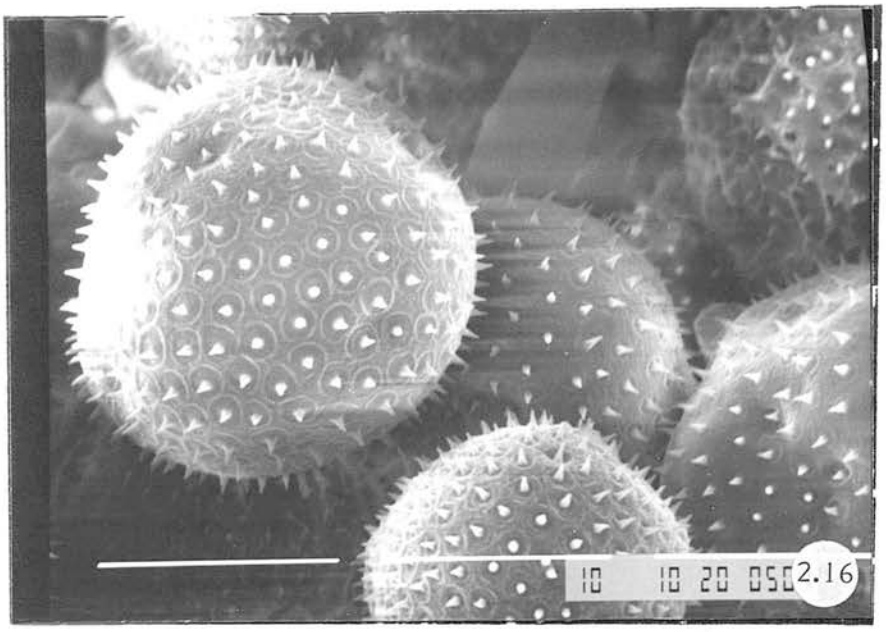
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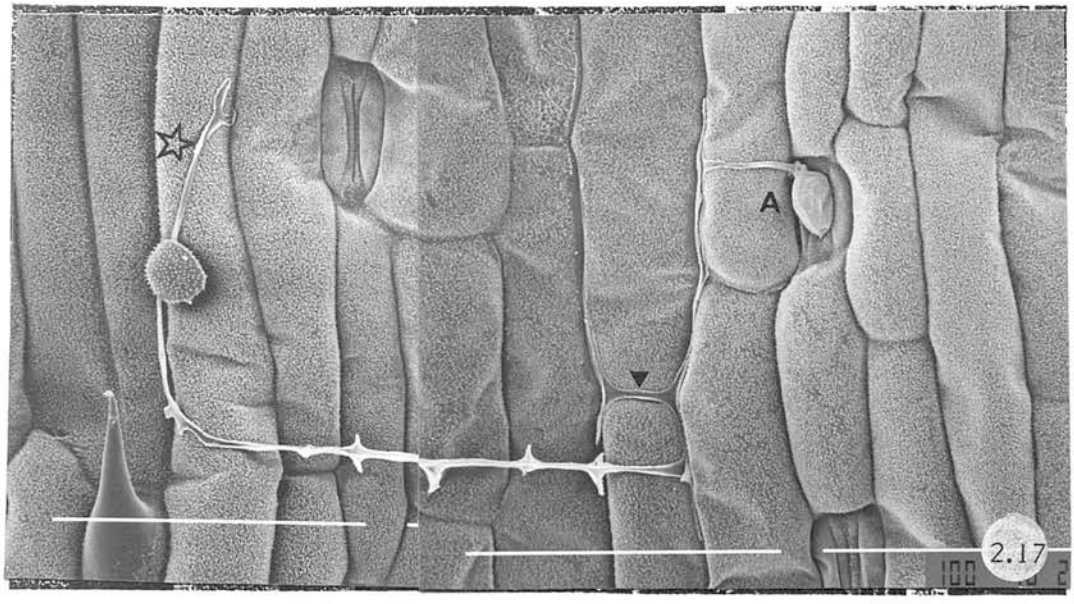
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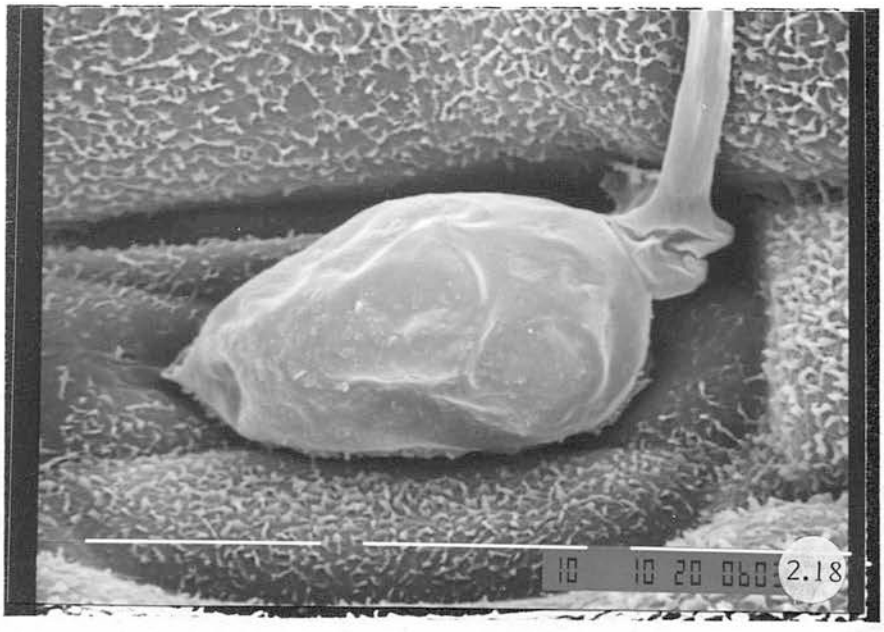
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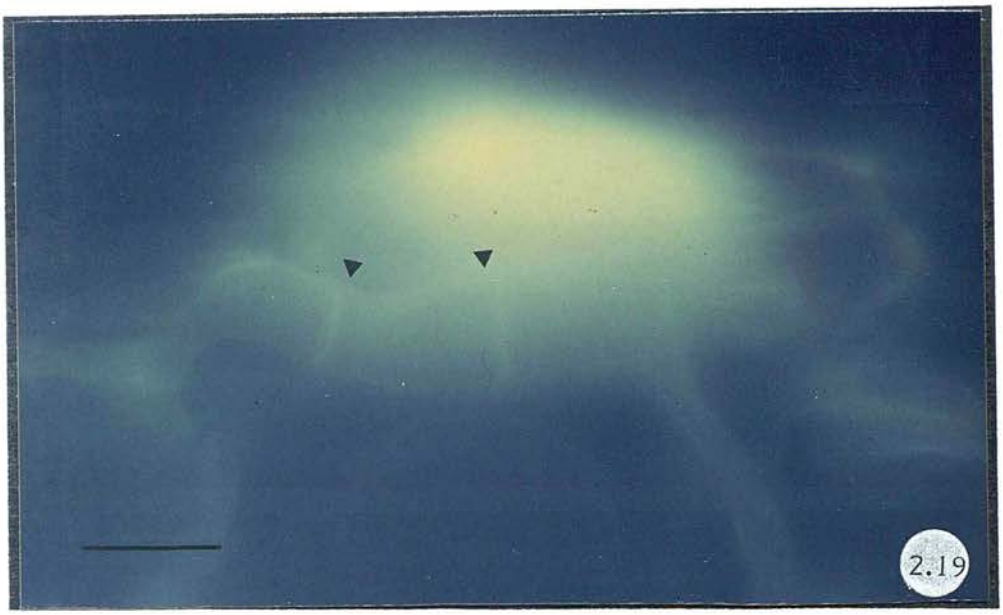
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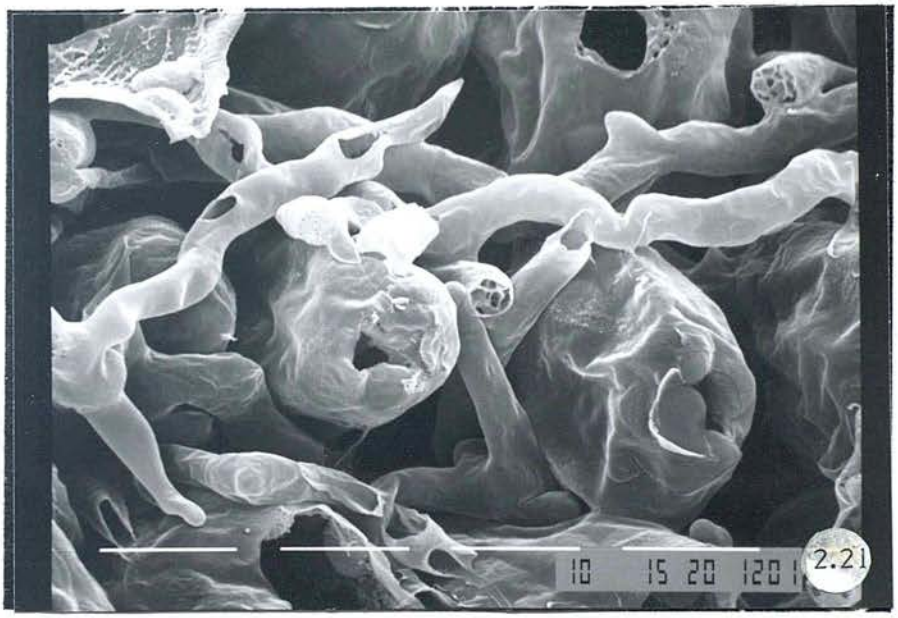
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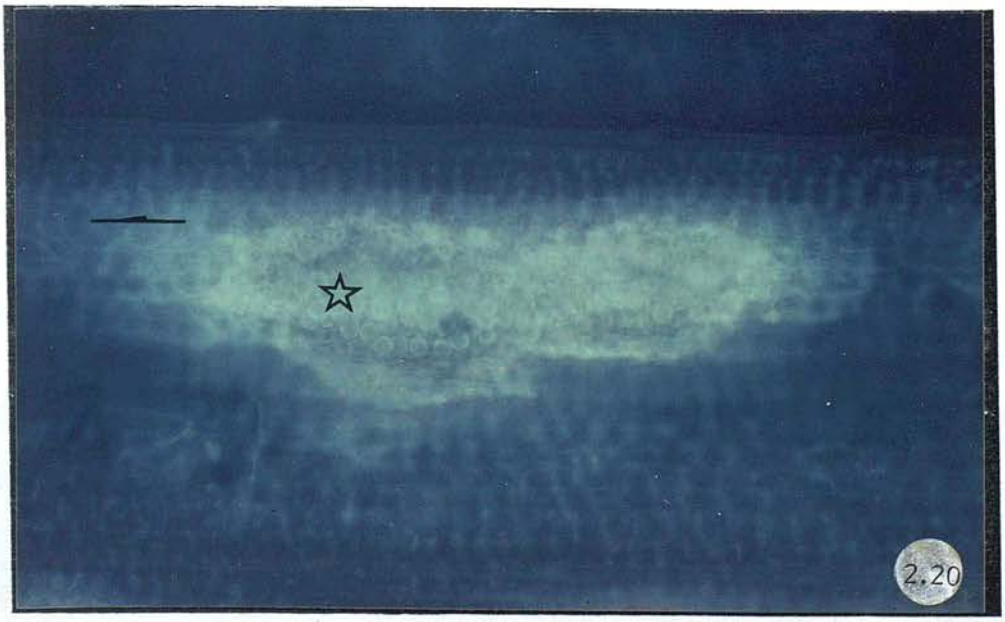
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simple paraphyses could be observed and, at the edge of each sorus, hyphae protruded occasionally from the mycelium (Plate 2.13) as was the case with P. coronata. The spores were relatively small in size and oval or round in shape (Plate 2.14), each with six to eight germ pores (Plate 2.15). The echinulations of the spores were relatively wide with small circular rings surrounding each spine (Plate 2.16). The germ tubes of barley brown rust were relatively short and always branched, with the main branch normally leading to the formation of a large appressorium on the host's stoma (Plates 2.17 & 2.18). Substomatal vesicles were longitudinal and septate, with normally three to four cells (Plate 2.19). Puccinia hordei grew slowly to form a dense mycelium (Plate 2.20) which can be seen surrounding the sorus in Plate 2.21. Hyphae had a medium diameter and produced many haustorial mother cells (Plate 2.22). Only occasionally did secondary sori arise beside the initial sorus (Plate 2.23). Green island formation was a very common phenomenon on barley hosts (Plate 2.24).

Puccinia recondita gave scattered dark brown pustules on rye (Plate 2.25). The sori reached 0.8 X 2.7 mm and were the biggest within

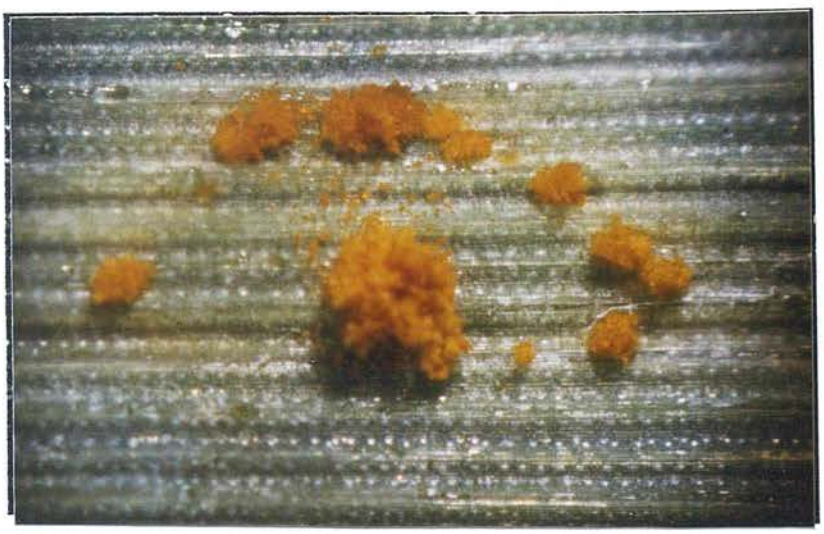


Plate 2.25 Urediniosori of P. recondita on rye; LM; X 10.

Plates 2.26 - 2.32 P. recondita.

Plate 2.26 Paraphyses at the sorus edge; young, immature spores (*);
SEM;
length of bar = 10 µm.

Plate 2.27 Mature urediniospore;
SEM;
length of bar = 10 µm.

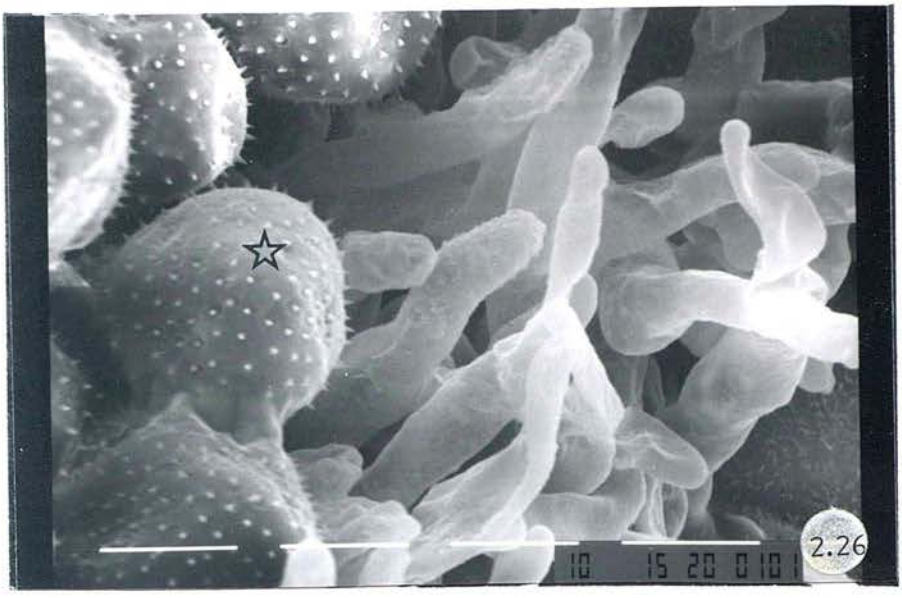
Plate 2.28 Germinating urediniospores; germ tube branching dichotomously (◄);
LM; live, unstained;
length of bar = 50 µm.

Plate 2.29 Young infection on rye; spore (*) and appressorium SSV (◄); bright staining cells are HMC;
FM; calcofluor stain;
length of bar = 100 µm.

Plate 2.30 Substomatal vesicle; septa (◄); infection hyphae (IH);
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.31 Haustorial mother cell showing haustorial neck (◄);
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.32 Secondary urediniosori around initial sori (two initial infections); 20 days after inoculation;
X 10.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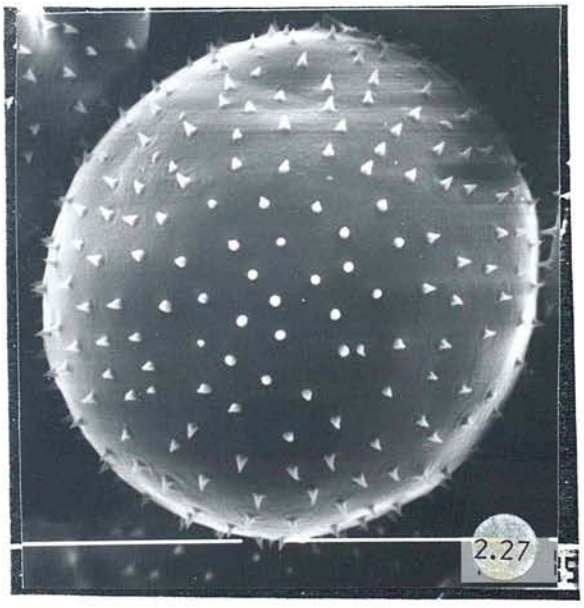
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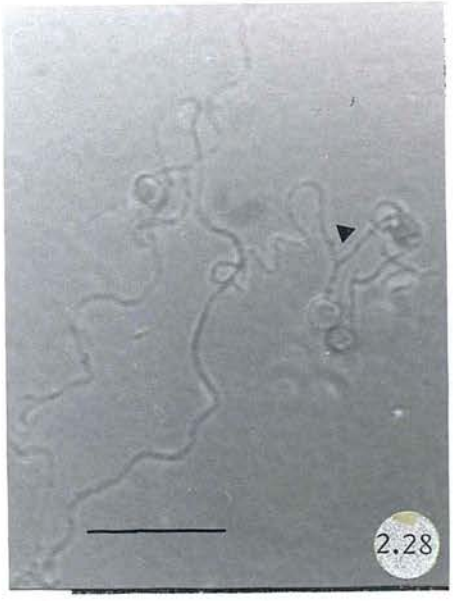
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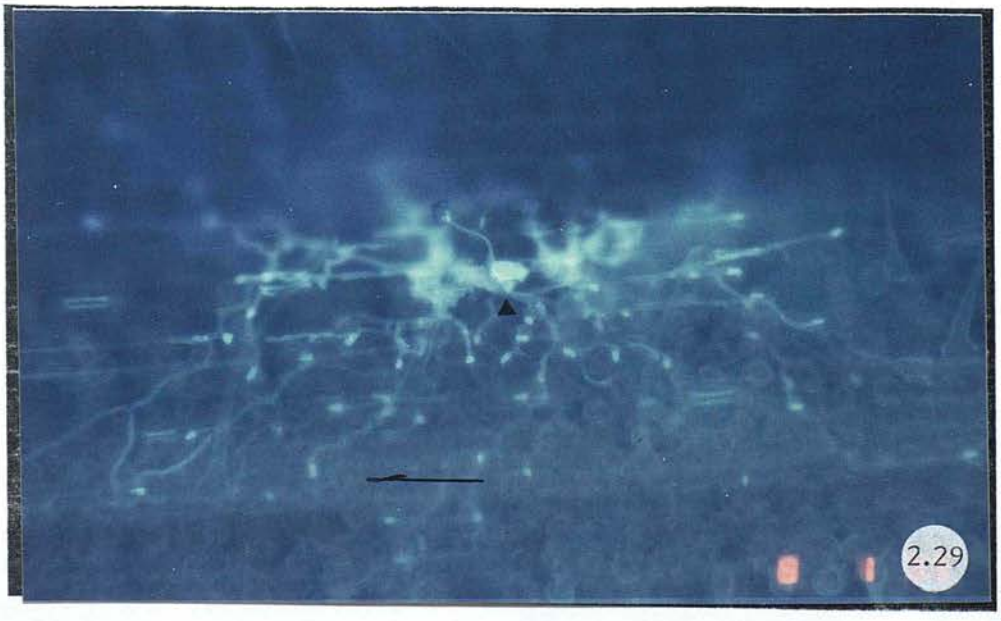
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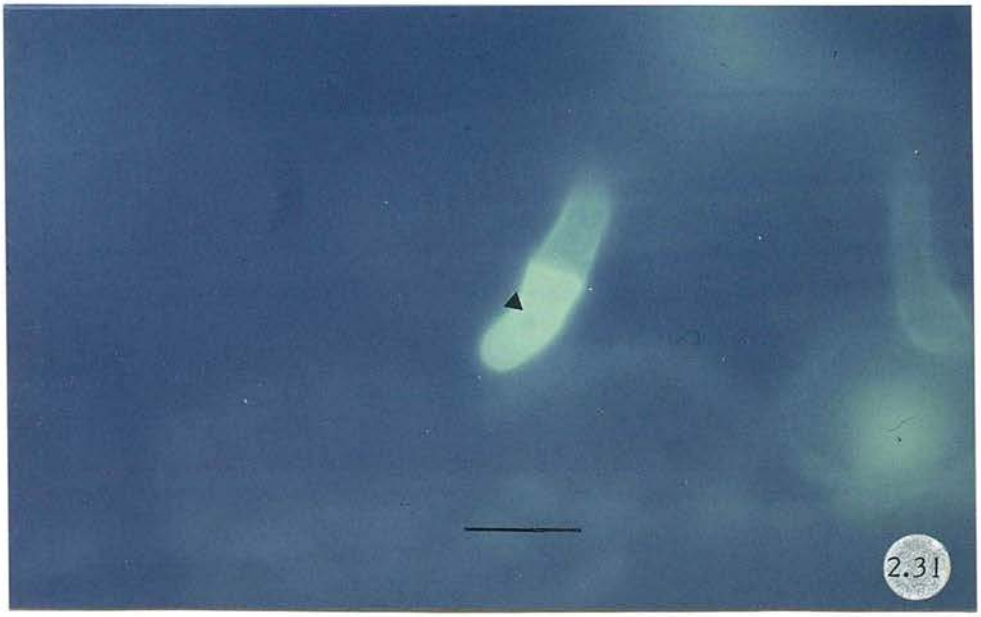

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the cereal leaf rusts. In some combinations many simple paraphyses could be observed (Plate 2.26). The spores were relatively big, with six to eight germ pores and their echinulation pattern was the widest within the rusts observed in this study. The spines were again surrounded by small circular ridges (Plate 2.27). Rye brown rust germ tubes were found to be of medium length: they sometimes branched dichotomously (Plate 2.28). Appressoria developed on stomata (Plate 2.29) and long, septate, usually two-celled, substomatal vesicles were present (Plate 2.30). In rye leaves a loose mycelium grew at a medium speed. Intercellular hyphae were relatively thick. The density of haustorial mother cells was low, partly because rye tissue possessed larger intercellular cavities than the other cereal hosts. Haustorial mother cells were short and often showed a dark spot when stained with calcofluor, presumably where the haustorial neck was formed (Plate 2.31). The initial sorus was often surrounded by a ring of secondary sori a short time after its eruption (Plates 2.25 & 2.32).

Puccinia striiformis on barley and wheat developed large colonies in host leaves. The sori were small but joined together longitudinally when the infection matured, forming long and narrow stripes of a yellow

Plate 2.33 Urediniosori of P. striiformis on wheat; LM; X 15.

Plates 2.34 - 2.39 P. striiformis.

Plate 2.34 Mature urediniospores of WYR;
SEM;
length of bar = 10 µm.

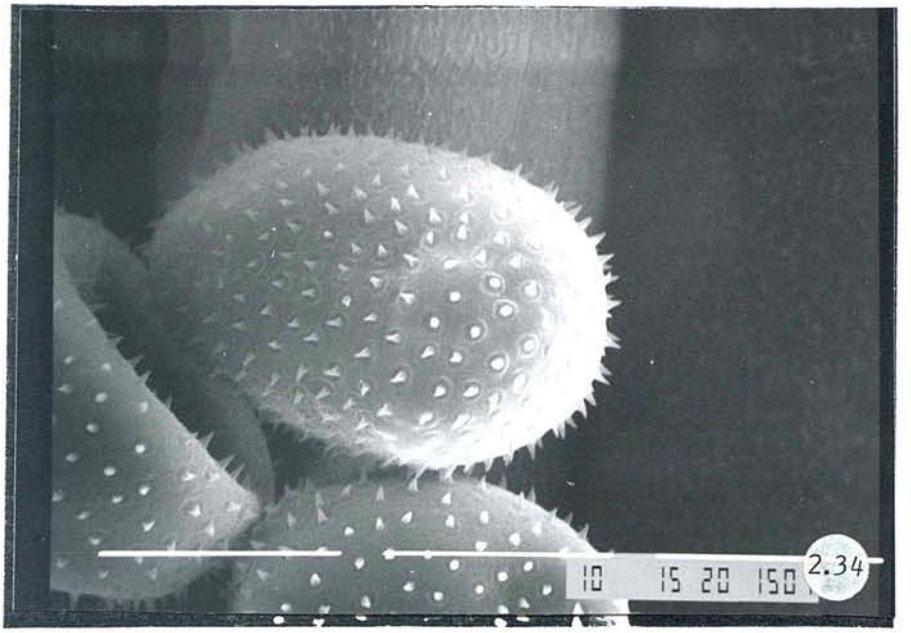
Plate 2.35 Young urediniospores of BYR; note echinulation and circular ridges;
SEM;
length of bar = 10 µm.

Plate 2.36 Germinating urediniospore of BYR on barley leaf; note the failure to penetrate the first three stomata encountered (◀) and the site of penetration (*);
FM; calcofluor stain;
length of bar = 100 µm.

Plate 2.37 Stomatal penetration of BYR on barley without appressorium formation;
SEM;
length of bar = 10 µm.

Plate 2.38 Formation of SSV (◀) and infection hyphae (*) in WYR; note also germ pores in spore (◁);
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.39 Runner hypha between host cells; BYR;
FM; calcofluor stain;
length of bar = 10 µm.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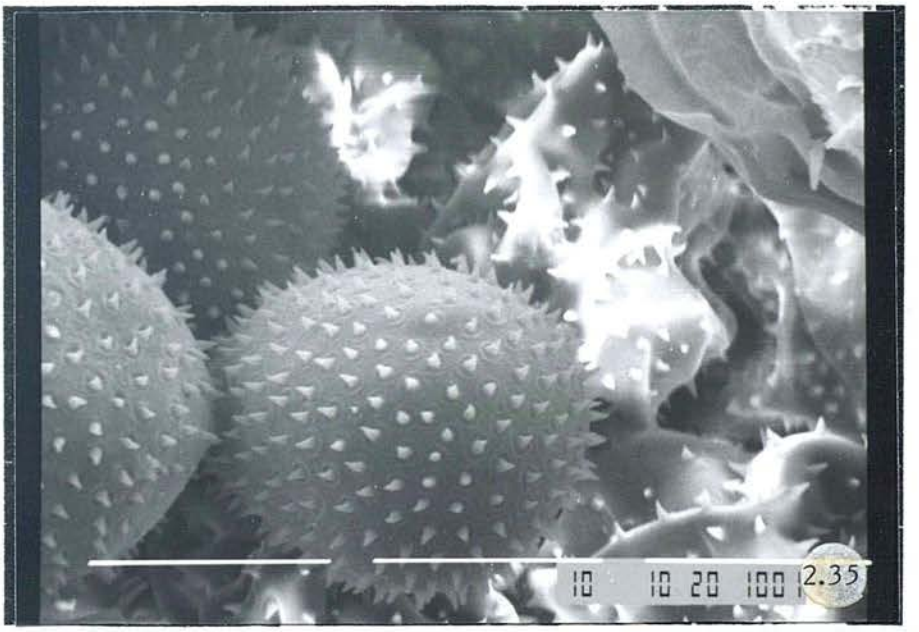
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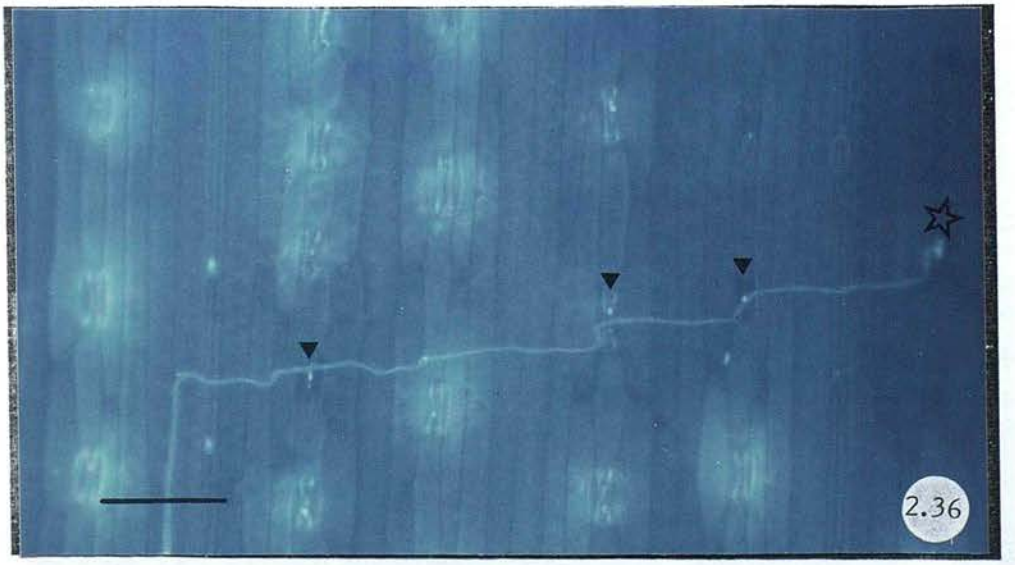
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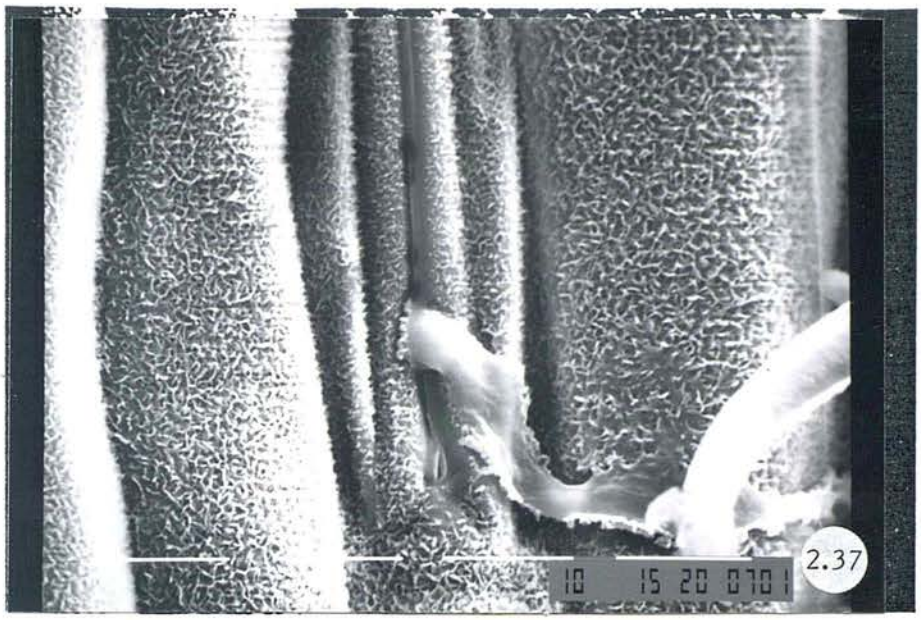
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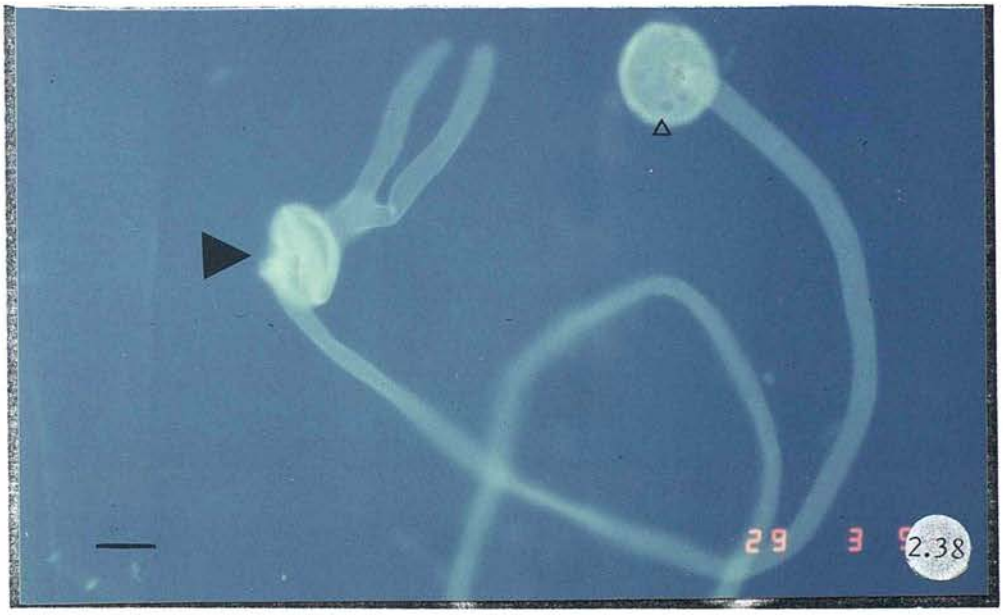
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to orange colour (Plate 2.33). Barley and wheat isolates showed no observable morphological differences but were not compatible with the respective other host. No paraphyses could be observed. The spores were of medium size and often pear shaped (Plate 2.34). The spore wall was hyaline, so that the cytoplasm determined the colour. Echinulation was relatively dense and the spines were again surrounded by circular ridges (Plate 2.35). The number of germ pores was seven to nine (to 14). Yellow rust germ tubes were very long and unbranched (Plate 2.36). They entered the stomata of hosts directly without forming appressoria (Plate 2.37). Inside the substomatal cavity a globular vesicle was formed with normally two or three infection hyphae branching off (Plate 2.38). In the host leaves P. striiformis grew very fast and developed a dense mycelium. The intercellular hyphae were large in diameter and runner hyphae of even bigger dimensions grew for a long stretch along the leaf veins (Plate 2.39). Haustorial mother cells were short and thick (Plate 2.39).

Puccinia triticina, the brown leaf rust of wheat, produced dispersed, chestnut brown pustules of medium size (Plate 2.40). Paraphyses and hyphae often protruded from the sori, especially at the

Plate 2.40 Urediniosori of P. triticina on wheat; LM; X80.

Plates 2.41 - 2.48 P. triticina.

Plate 2.41 Simple paraphyses at the sorus edge;
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.42 Lobed paraphyses together with simple ones at the sorus edge;
SEM;
length of bar = 10 µm.

Plate 2.43 Mature urediniospore; note the shape of circular ridges;
SEM;
length of bar = 10 µm.

Plates 2.44 - 2.46 Appressoria and SSV.
Plate 2.44 Formed outside the host leaf; germ tube (GT), appressorium (*), SSV (◄) and infection hyphae (IH);
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.45 Formed in host leaf; grm tube (GT), appressorium (*), SSV (◄) and infection hyphae (IH);
FM; calcofluor stain;
length of bar = 50 µm.

Plate 2.46 Appressorium (*), SSV (◄) and infection hypha (IH) on host leaf;
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.47 Deep focus of infection site; infection growing into depth of leaf before spreading laterally; infection hyphae (IH);
FM; calcofluor stain;
length of bar = 10 µm.

Plate 2.48 Medium dense mycelium spreading the whole depth of the leaf; adaxial stomata (◄), abaxial stomata (◁); bright staining cells are HMC;
FM; calcofluor stain;
length of bar = 100 µm.


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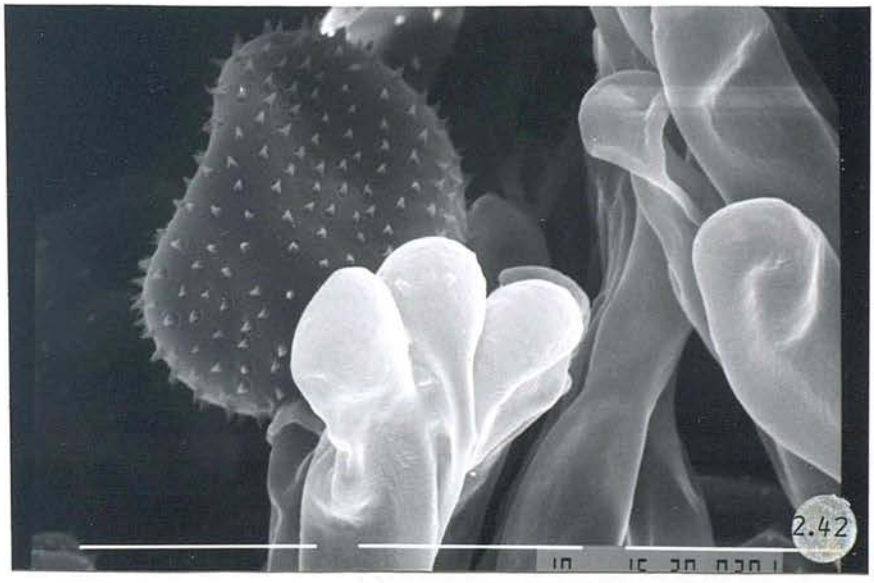
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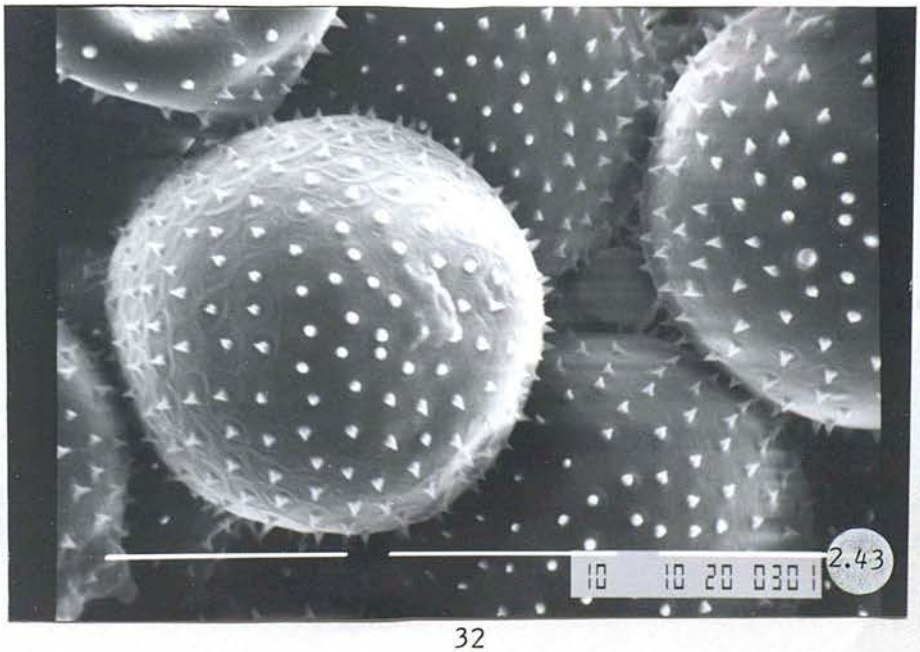
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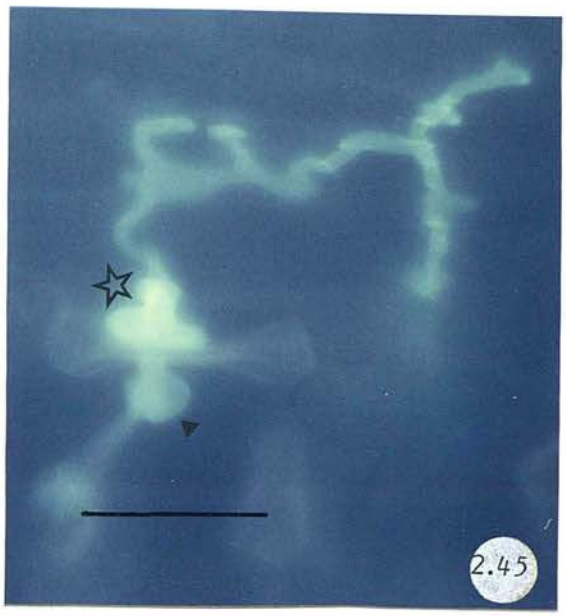
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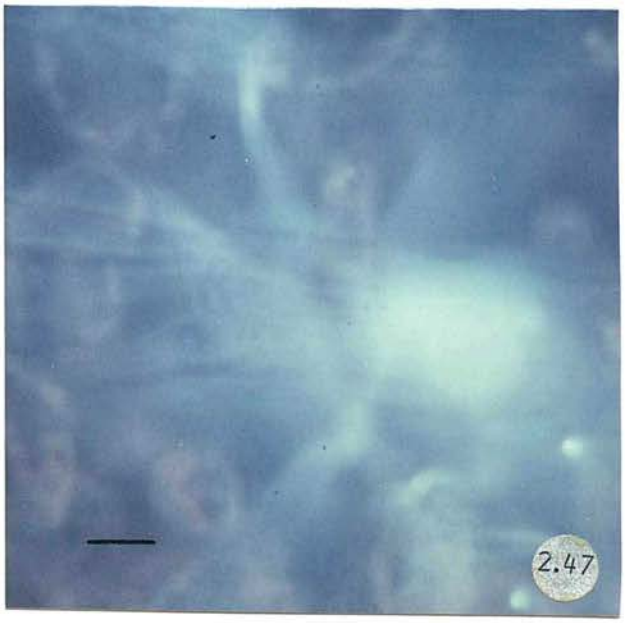
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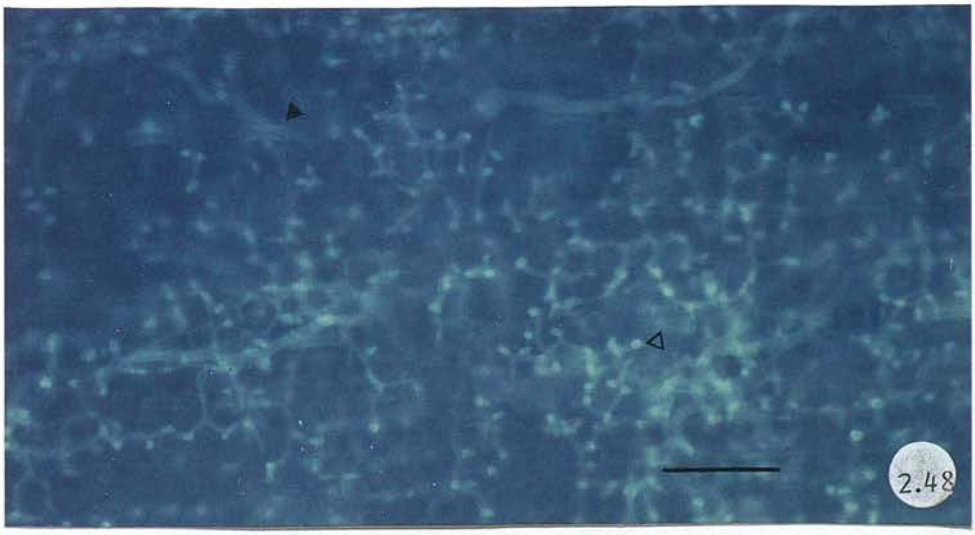

2.48

sorus edges (Plate 2.41) and sometimes paraphyses showed special shapes (Plate 2.42). The urediniospores were relatively large and the distance between neighbouring spines of the spore echinulation was small. Each spine was surrounded by a circular ridge, but sometimes these ridges joined to form a net-shaped structure (Plate 2.43). The number of germ pores per spore varied from seven (six) to nine. Germ tubes of the wheat brown rust were of medium length. They were unbranched when germinated on agar (Plate 2.44), but formed small branches on wheat leaves (Plate 2.45). Appressoria were formed on host stomata and globular substomatal vesicles with four to five infection hyphae were produced (Plates 2.44, 2.45 & 2.46). The rust developed at a medium speed. The colonisation of the host tissue took place by sending infection hyphae deep into the mesophyll (Plate 2.47) rather than spreading mainly close under the epidermis, as the other leaf rusts did, and a dense mycelium grew through the whole depth of infected leaves (Plate 2.48). Infection hyphae were relatively narrow with haustorial mother cells of a medium size.

Discussion:

The present results show clear morphological differences over a wide range of morphological characters between the investigated cereal leaf rusts in their uredinial spore stage. In the past only one worker, Pole Evans (1907), brought into consideration features such as the morphology of appressoria or substomatal vesicles for taxonomical purposes. Other workers (Gaeumann, 1959; Wilson & Henderson, 1966; Urban, 1967; Cummins, 1971; Bartos, 1984 and Savile, 1984) mainly emphasised features like spore size or host range to establish the taxonomy of cereal rusts.

The sorus size is mentioned by some workers (Pole Evans, 1907; Gaeumann, 1959; and Wilson & Henderson, 1966), but it seems that this feature is partly determined by the host plant tissue and by environmental conditions. The variation within the species is relatively great, and only part of any interspecific differences may be attributed to the fungal genotype. In yellow rust the sorus size is limited by the relative proximity of neighbouring sori which is a result of the semi-systemic colonisation and the rapid growth of this fungus.

In parasites like the brown rusts which produce scattered pustules and grow at a slow or medium speed, relatively large pustule sizes are characteristic: the large size giving large numbers of spores may be regarded as compensating for the fewer sori in comparison with yellow rust where large numbers of spores arise by the production of fast growing colonies of unlimited size with many sori from single infections (see Chapter 5).

Although the presence of paraphyses in urediniosori has been considered unimportant taxonomically (Grove, 1913), this feature was included in the present study. Possibly only one of the earlier descriptions of cereal rusts (Arthur, 1934) mentioned paraphyses in the sori of P. recondita and P. triticina, while variable observations appear in the literature on other rusts. Cummins (1971) and Savile (1984) report few or no paraphyses for P. coronata, P. hordei and P. striiformis; Gaeumann (1959) and Wilson & Henderson (1966) report the presence of paraphyses in P. coronata only. From the present study, simple paraphyses, which were observed within sori of all species, probably represent non-functional spore pedicels which occur in certain environmental conditions only. Together with the sterile hyphae protruding at the periphery of the sorus they can separate the spore mass and

promote spore detachment in damp conditions. More distinct paraphyses, as in WBR, however, seem to represent features of greater importance. Hiratsuka & Sato (1982) described several types of urediniosori in relation to their taxonomic position and the cereal leaf rusts can be classed into two of these categories: aparaphysate and capitate paraphysate. Their form of classification, however, would seem to be too rigid and, from the evidence of the present work, all cereal rusts were found to develop simple paraphyses like structures, at least in certain conditions, and P. triticina was approaching a capitate paraphysate form.

Another character of minor taxonomical importance is the spore colour. As it varies from isolate to isolate and colour mutants of brown and yellow rusts have been reported (Watson & Luig, 1968; Nayar et al., 1981; Newton et al., 1981), some caution is needed in its interpretation. However, as a first differential character, the spore colour can be useful in the field. In yellow rust the spore wall is completely devoid of pigment and the orange colour originates from the cytoplasm. The cytoplasm itself is thus unprotected against damage through radiation. This may be one of the reasons why, in singie spore inoculations, yellow rust performed less successfully than the other leaf rusts. In the other rusts the brown pigment is located in the spore wall, and the cytoplasm has a light orange colour. This could provide protection against radiation damage.

The spore size depends much on the water contents of the spores and the methods of examination. The present investigations showed that spores on water agar were up to 50% bigger in diameter than vacuum dried spores. Also, the method of preparation and observation influence the spore size greatly (Beckett et al., 1984), although the method of

preparation and examination is not always mentioned in the literature: observations in lactophenol were carried out by a number of investigators (Cummins, 1971; Savile, 1984; Pole Evans, 1907). The spore sizes reported by these and other workers were generally bigger than the results from this study, but results of individual workers differ. The relative sizes of spores of the different rusts, however, follow roughly the same trend, although slightly different rankings are evidenced (Table 2.2). The importance of the spore size for the survival of the fungus can be viewed in two ways: larger spores contain more reserve material and thus give the fungus an advantage in possibly longevity and in the first stages of infection. On the other hand more material is invested into fewer propagules, and the transportation in air currents and the epidemic spread of bigger spores is less extensive.

The echinulation pattern of the rusts in this study was closely linked to the spore sizes, where the number of spines on every spore is roughly the same and the distance between spines becomes a variable of the spore size. Only since the development of the electron microscope can precise measurements be made, although Fischer (1904) and more recent workers (Gaeumann, 1959; Wilson & Henderson, 1966; Savile, 1984) tentatively described this feature from light microscopic studies. The presence and shape of circular ridges (annuli) is partly due to remains of degraded external wall layers of the spores. The spines develop beneath the primary wall of immature spores and are pushed to the surface by subsequent deposition of wall material from the plasmalemma (Amerson & Van Dyke, 1978; Littlefield & Heath, 1979). The primary outer wall breaks open and erodes. The formation of net-shaped structures as in P. triticina might have its origin in the relative

Table 2.2
Comparison of spore size measurements in the literature

Literature source	Rust isolates *) (Length X breadth or diameter in µm) OCR	BBR	RBR	YR **)	WBR
Fisher (1904)	14-18 X 16-21	19-22 X 22-27	20-28	15-20 X 17-30	19.2-27.2
Sydow (1904)	16-24 X 20-30	15-20 X 22-27	22-28')	18-26 X 25-30	22-28')
Pole-Evans (1907)	20-22	17-22 X 20-30	19-20	25-30	19-20 X 24-25
Liro (1908)	17-24	19-24	20-28 X 28-34	20-30	19-27
Grove (1913)	14-20 X 16-25	16-28')	16-28')	18-26 X 25-30	16-28')
Fragoso (1924)	14-18 X 16-21	18-22 X 18-27	22-26 X 22-28	15-20 X 17-30	20-27
Arthur (1934)	16-20 X 18-24	18-24 X 22-28	13-24 X 16-32')	16-26 X 19-30	13-24 X 16-32')
Savulescu (1953)	14-20 X 15-25	18-24 X 20-30	17-23 X 20-28	15-26 X 17-30	18-24 X 20-28
Gaeumann (1959)	17-21 X 24-27	17-22 X 20-30	17-22 X 20-28	13-23 X 14-36	17-22 X 18-29
Wilson & Henderson (1966)	10-35 X 13-39	15-20 X 18-26	13-24 X 16-34')	12-24 X 25-30	13-24 X 16-34')
Cummins (1971)	20-24 X 25-30	18-25 X 21-30	20-25 X 24-32')	20-24 X 25-30	20-25 X 24-32')
Savile (1984)	17-23 X 22-29	18-25 X 23-30	21-27 X 25-29	18-24.5X26-30	19-24.5X22-30
Present results	16 X 22	17 X 23	19 X 23	17 X 22	18 X 23

*) O = Oats CR = Crown Rust
B = Barley BR = Brown Rust
R = Rye YR = Yellow Rust
W = Wheat

**) Wheat and Barley Isolates

') considered as the same species in publication

proximity of the spines. The function of the urediniospore echinulation is to facilitate the spore's attachment to host leaves and possibly the attachment of spores to one another, as in P. striiformis. Some advantage may lie in increased friction when air-borne.

Apart from one exception (OCR), where the number of germ pores varies considerably in the literature, this feature is reported consistently by all workers. During germination all the germ pores swell, but the germ tube is finally formed from only one of them. Occasionally two germ tube initials protrude from the spore during the first hours of germination, but when the main germ tube growth started only one tube remained (Plate 2.17; see also Chapter 5).

The branching characteristics of rust germ tubes represent an adaptation to the surface topography of their hosts' leaves (Dickinson, 1970; 1971; 1972). From the presented data it can be seen that two strategies have evolved to obtain maximum efficiency in finding stomata: firstly, germ tubes can grow for a long way across the leaf blade without branching. Sooner or later they will come across one of the host's stomata which are arranged in parallel rows along the leaf. This strategy was found in P. coronata, P. striiformis and P. triticina. Secondly, the germ tubes can branch in the proximity of the germinating spore to find the nearest stoma as could be observed with P. hordei and P. recondita. According to the literature, cereal rust germ tubes do react to chemotropic stimuli (Grambow & Reisener, 1976) but the host leaf cuticle also provides a thigmotropic stimulus for the growth across the leaf (Johnson, 1934; Dickinson, 1949; Lewis & Day, 1972; Staples & Macko, 1984). The germ tubes of all cereal leaf rust

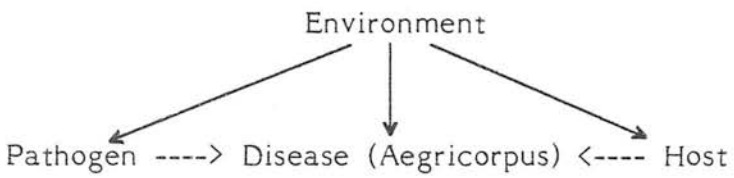
fungi examined in this study grew across the leaf blade, some short and branching (P. hordei and P. recondita) others long and unbranched (P. coronata, P. striiformis and P. triticina).

CHAPTER 3

Culture of cereal leaf rusts

Introduction

When studying rust fungi and other obligate parasites special difficulties arise from the complicated relationship between the pathogen, the host, the disease and the influence of environmental conditions on all three factors. Loegering (1984) described a model of this relationship, reproduced below:



One approach towards an understanding of this system is to investigate the physiological requirements and reactions to environmental changes of each partner, i.e. host and parasite, separately. This necessitates growing the partners in isolation. The growth of the host partner in controlled, aseptic conditions is relatively easy and has been described many times (see for example Metzler, 1981; Chares et al., 1983). The growth of rust fungi in axenic conditions, however, has been difficult: although early experiments with the culture of spermatia (Plowright, 1889) were successful, they could never be reproduced until recently (Deml et al., 1982a, b). Until the early 1950s, experiments with other stages in the life cycle of the rusts, such as those of Fuchs and Gaertner (1958), were always abandoned in failure, although

Colley (1918) and Wright (1977) reported the continuing growth of promycelium from germinating teliospores of Cronartium ribicola and P. striiformis respectively. Hotson & Cutter (1951) reported the growth of Gymnosporangium juniperi-virginianae in tissue cultures of rust galls from Juniperus, and the subsequent axenic growth of the pathogen apart from the host tissue. Although this early success in axenic culture was not confirmed in a later paper (Hotson, 1953), and considerable controversy arose about its validity (Scott & Maclean, 1969), Cutter (1959) confirmed the earlier findings and extended them to other rust. Meanwhile, other workers maintained dual tissue cultures of infected hosts (Bauch & Simon, 1957; Turel & Ledingham, 1957), where the rust was always dependent on living host tissue.

A major breakthrough in the axenic culture of rusts was achieved when Williams et al. (1967) succeeded in establishing growth with densely sown urediniospores of P. graminis tritici on relativly simple media containing yeast extract and peptone. Several researchers had concentrated on this spore form as a basis for axenic culture before, but mostly single spores or low spore densities had been used, to facilitate the observation of the process of transition from the germ tube stage to saprophytic growth (Arthur, 1928; Stock, 1931; Fuchs & Gaertner, 1958; Gaertner & Fuchs, 1962): no continuous culture of rusts had been obtained. Since 1967 many rust isolates have been taken in axenic culture starting from urediniospores. The literature of this work has been reviewed by Scott & Maclean (1969), Scott (1976), Maclean (1982) and recently in a splendid review by Williams (1984).

Different aspects of axenic growth which were studied were: a) the development of culture methods; b) the induction of sporulation in culture; c) the pathogenicity on the original host of an isolate in

axenic culture; and d) the nuclear condition of cultures. Table 3.1 lists the most prominent literature on axenic culture of rust fungi up to the present day.

The early enthusiasm about axenic culture of rusts has slowed down in the recent past, as the application of the results of physiological studies *in vitro* on host-parasite interactions *in vivo* could only be made with great reservation. It was, therefore, necessary to approach the questions concerning host-parasite relationships from a different angle also.

In the diagram shown earlier it is indicated that the environment plays an important role in disease development, influencing all three organisms (*sensu* Loegering, 1984) in the relationship, i.e. host, parasite and aegricorpus. It is therefore important to provide environmental conditions as uniform as possible when the effects of host and parasite on the development of disease are being studied. One way of providing uniform conditions is the raising of host plants in controlled environment cabinets, where temperature, humidity and light cycle and intensity can be controlled. This is costly and requires large amounts of space and equipment, especially if different rust isolates are being tested simultaneously and have to be isolated from each other to avoid cross contamination. Another way of providing constant environmental conditions is to carry out the experiments with rusts on the detached leaves of their respective hosts, the leaves being laid out in petri dishes on a suitable medium to prevent wilting and senescence. The first attempts to grow rusts on detached host leaves in petri dishes were made by Clinton & McCormick in 1918 with *Cronartium ribicola* on *Ribes nigrum* (Clinton & McCormick, 1924). They simply used water to keep the leaves fresh. Yarwood (1946) gives a good account of

Table 3.1 Summary of the historical development in methods of axenic culture of rust fungi.

RUST	EXPERIMENTAL NOTES	RESULTS	REFERENCE
1. Several species	yeast budding from spermatia	yeast colonies	Plowright (1889).
2. Gymnosporangium juniperi-virginianae	culture methods from infected callus	mycelium growth	Hotson & Cutter (1951), Cutter (1959)
3. Uromyces ari-triphylli	culture methods from infected callus	mycelium growth	Cutter (1952; 1960a).
4. Puccinia malvacearum	culture methods from infected callus	mycelium growth	Cutter (1960b).
5. Puccinia graminis f.sp. tritici	culture methods from urediniospores	growth of aerial mycelium	Williams et al. (1966), Bushnell (1968), Coffey et al. (1969), Bushnell & Rajendren (1970), Wong & Willets (1970), Bushnell & Stewart (1971), Hartley & Williams (1971), Kuhl et al. (1971), Green (1976).
	defined medium	mycelium growth	Foudin & Wynn (1972).
	growth rates; physiological races	differential growth	Bushnell (1976).
	physiology; growth stimulation	effects of DTT or BIM *) on growth	Fry & Willets (1974), Grambow & Mueller (1978).
	pathogenicity	change of host range	Williams et al.(1967), Maclean & Scott (1974).
	cytology, mycelium, spores	monokaryotic mycelium, diploidy	Williams (1971), Williams & Hartley (1971), Maclean et al. (1974), Grambow & Mueller (1978).

Table 3.1 continued

RUST	EXPERIMENTAL NOTES	RESULTS	REFERENCE
6. P. graminis avenae	culture methods from urediniospores	aerial mycelium	Kuhl et al. (1971), Green (1976).
7. P. graminis secalis	culture methods from urediniospores	no aerial mycelium	Kuhl et al. (1971), Green (1976).
8. P. coronata avenae	culture methods from urediniospores	no aerial mycelium	Kuhl et al. (1971), Green (1976).
	pathogenicity, sporulation	reinfection of host	Ando & Katsuya (1982).
9. P. triticina	culture methods from urediniospores	prolonged growth	Kuhl et al. (1971), Green (1976), Raymundo & Young (1974).
	sporulation, abnormal spores	mesospore formation	Ando & Katsuya (1979).
10. P. helianthi	culture methods from urediniospores	aerial mycelium	Coffey & Allen (1973).
11. P. striiformis, barley	culture methods from urediniospores	very small colonies	Williams (1976).
12. P. horiana	culture methods from infected callus	mycelium & spores	Ando et al. (1979).
13. Coleosporium tussilaginis	culture methods from spermatia	monokaryotic yeasts	Deml et al. (1982a,b).
14. Melampsora lini	culture methods from urediniospores	aerial mycelium & urediniospores	Turel (1969), Quick & Cross (1971), Lane & Shaw (1972), Coffey & Allen (1973).
15. Phragmidium mucronatum	culture methods from urediniospores	mycelium & spores	Bhatti & Shattock (1980).

*) DTT = dithiothreitol BIM = 3-3´-bis indolemethane

the methods used and the results obtained by earlier researchers. More advanced methods were used later with various concentrations of benzimidazole (Person et al., 1957; Samborski et al., 1958; Bjoerkmann, 1960; Lumbroso et al., 1977) or of other chemicals with similar effects (Wang et al., 1961; Wolfe & Macer, 1964).

In this section consideration is given to preliminary investigations aimed at establishing methods of culturing rust fungi for the experimental studies. Two studies were carried out as follows:

(a) Axenic culture,

(b) Detached leaf culture studies.

Materials and methods

a) Axenic culture studies

For the axenic culture experiments the culture media described by Bushnell (1968) were employed (Appendix 3.1). Spores from urediniosori of P. striiformis or P. coronata, on leaves surface sterilised with 70 % ethanol for 2 min. and subsequently rinsed in sterile distilled water just prior to sorus eruption, were inoculated singly and in clusters on the agar surface and incubated at different temperatures. P. striiformis isolates were incubated at 4 °C in the dark, and at 14 or 22 °C, in a 16 h light / 8 h darkness regime. P. coronata was incubated at the two higher temperatures only and in the same light / darkness regime. An additional experiment was carried out with P. striiformis teliospores: leaf sheaths of barley bearing yellow rust teliosori were surface sterilised in 20 % hydrogen peroxide for 5-10 min, rinsed twice in sterile distilled water, cut into very small segments and incubated on Bushnell's culture medium at 14 °C. Some of the inoculations were carried out on agar covered microscope slides

which were then stained in Giemsa HCl (Appendix 3.2) for cytological examination.

The experiments were carried out with two replicates using 10 50 mm diameter, plastic petri dishes for each condition.

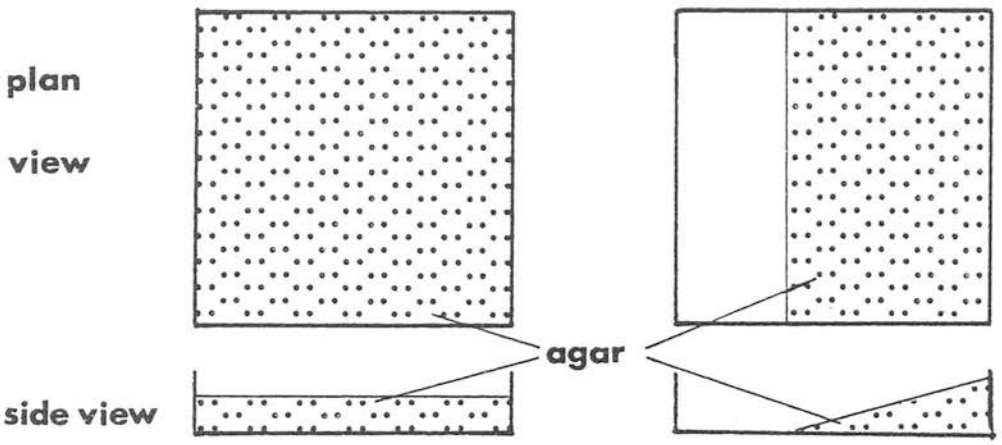
b) Detached leaf culture studies

Seedling leaves of cultivars of barley, oats, rye, triticale and wheat, grown in trays in a controlled environment, were used. The varieties which were employed are indicated in Appendix 1.1. The seedling leaves of the cereals were harvested when they had fully expanded, and in the middle of the light cycle, as it seems important that the translocation of carbohydrates is interrupted when a high concentration of assimilates is present in the leaves (Samborski et al., 1958). After detachment they were layed out without delay onto the surface of the various treatments in petri dishes. The light and temperature conditions of the seedlings before detachment were identical to the conditions of the detached culture. All experiments were carried out in sterile 100 mm square plastic petri dishes.

Three different factors were varied to find the most suitable conditions for maintaining cereal leaf rusts in detached leaf culture. i) The concentration of benzimidazole dissolved in the medium: four different concentrations were tried, 0 ppm, 40 ppm, 80 ppm and 100 ppm. ii) The supporting medium: three concentrations of water agar, 0.4 %, 0.7 % and 1.0 %, were poured in two different ways, horizontally and sloping (Fig. 3.1), and two other support media were used, cotton wool and filter paper. iii) Temperature and light regimes: temperatures of 10, 14 and 22 °C and light applied for 8, 16 and 24 h per day. The experiment was replicated five times.

The seedling leaves were inoculated with the appropriate rust

spores immediately after they had been transferred to the various culture media. A list of rust isolates used is given in Appendix 1.2.



a) horizontal agar b) sloping agar

Fig. 3.1 Two ways in which agar was poured.

Results

a) Axenic culture studies

None of the methods used led to continuing growth of axenic cultures of either rust. P. striiformis urediniospores formed unbranched and undifferentiated germ tubes in all conditions but germinated best at 14 °C and only very little at 22 °C: good germination could also be achieved at 4 °C. The germ tubes grew to a length of up to 2.0 mm. Nuclei could be found at a distance of between 30 and 160 µm from the germ tube tip (Plate 3.1). P. coronata germinated best at 22 °C but also very well at 14 °C. The germ tubes on agar were unbranched (Plate 3.2), undifferentiated and much shorter than in P. striiformis (0.8 mm): occasionally a "hypha" (Plate 3.3) of smaller size would branch off the germ tube and grow out to a considerable length. This "hypha", however, was never septate nor branched and did not lead to any further growth. The nuclei could be found at 30-160 µm

Plates 3.1 - 3.8 Axenic growth experiments with P. striiformis and P. coronata.

Plate 3.1 Germination of P. striiformis on nutrient agar; nuclei (◁);
LM; Giemsa stain;
length of bar = 10 µm.

Plate 3.2 Germination of P. coronata on nutrient agar; no differentiation occurred;
LM; live, unstained;
length of bar = 100 µm.

Plate 3.3 Germination of P. coronata on nutrient agar; primary germ tube (*) and hypha branching off (◀);
LM; live, unstained;
length of bar = 10 µm.

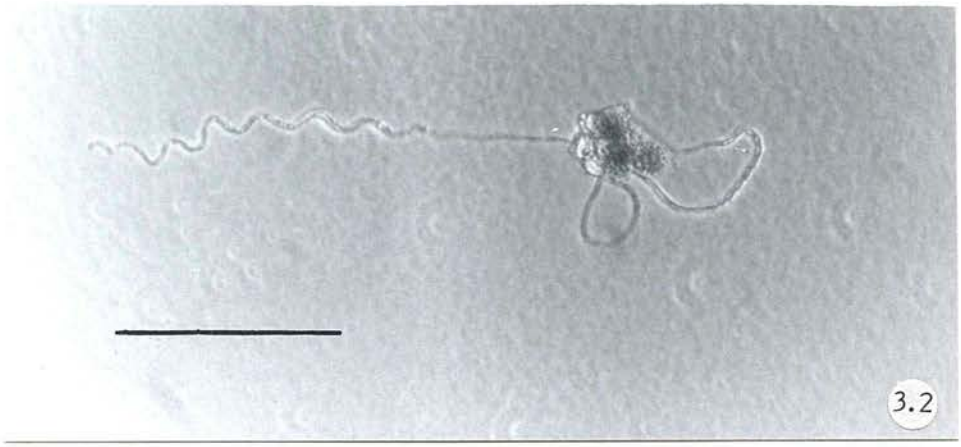
Plate 3.4 Basidiospores of P. striiformis BYR on sterigmata (◀);
LM; live, unstained;
length of bar = 10 µm.

Plate 3.5 As in Plate 3.4;
SEM;
length of bar = 10 µm.

Plates 3.6 & 3.7 Germinating basidiospores of P. striiformis BYR on nutrient agar; appressorium like structure (*);
LM; live, unstained;
length of bar = 5 µm.

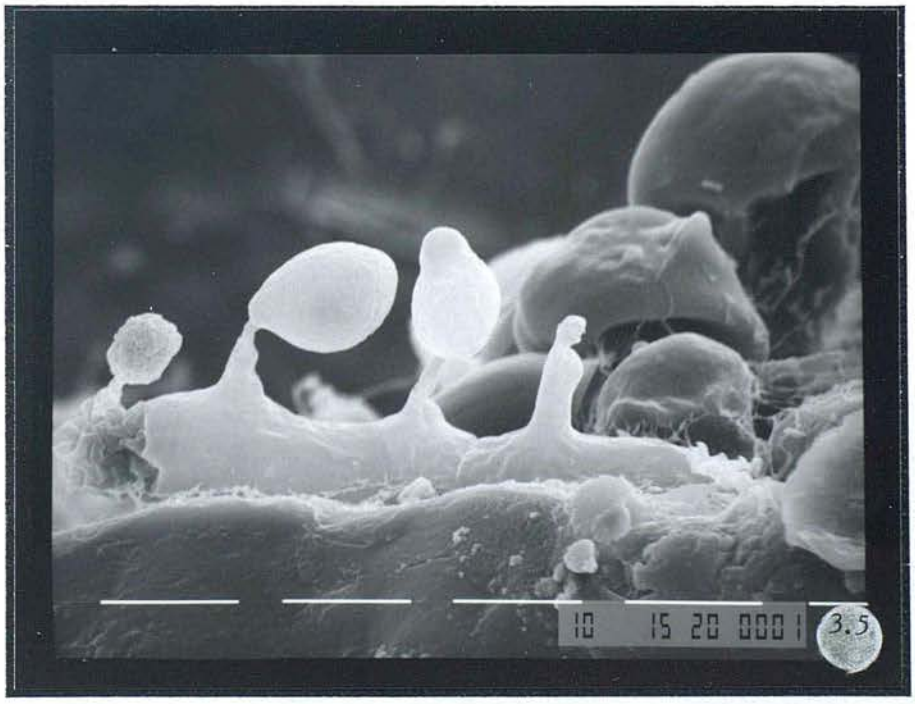
Plate 3.8 Binucleate basidiospore of P. striiformis;
LM; Giemsa stain;
length of bar = 5 µm.

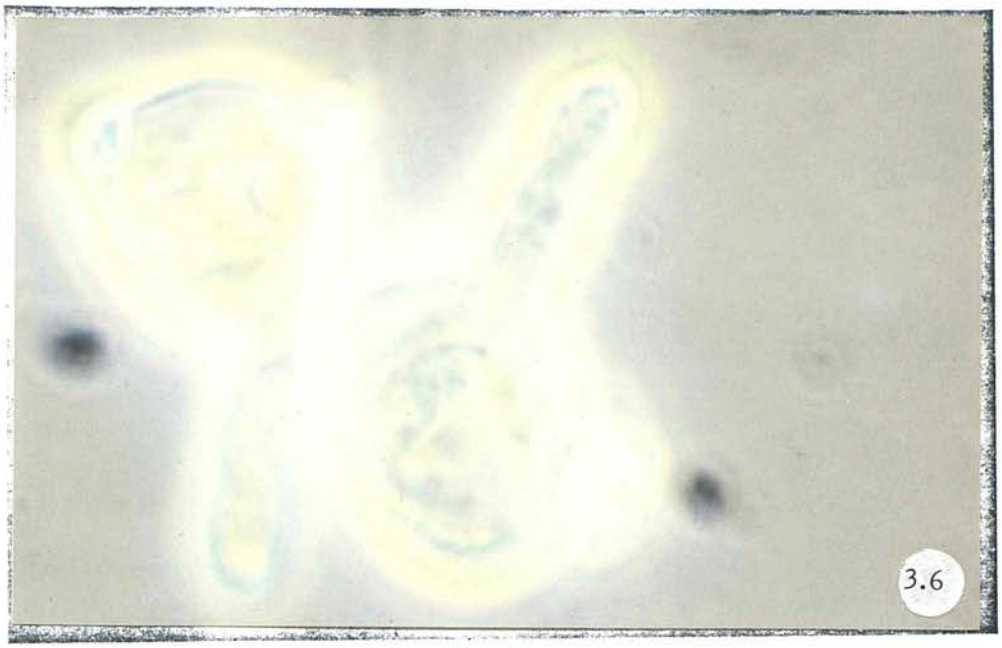
3.1



3.2

3.3




3.6


3.7


3.8

from the germ tube tip, as with P. striiformis, or in the "hyphae" in a similar position. In the present experiments all germ tubes and "hyphae" were dikaryotic and no mitosis could be observed.

In the experiments with teliospores of P. striiformis, germination started without delay and led to long promycelia in the agar without septation. After 24 h many basidia were formed outside of the agar, bearing four basidiospores on sterigmata (Plates 3.4 and 3.5). Once the basidiospores were released, they germinated for a short time on the agar and collapsed subsequently (Plates 3.6 and 3.7). Basidiospores were first uninucleate but became binucleate when mature (Plate 3.8). No axenic growth could be established.

b) Detached leaf culture studies

The results for rust development are summarised in Table 3.2. In the benzimidazole experiment, the highest concentration (100 ppm) preserved the leaves best, but very good preservation was also achieved with 80 ppm benzimidazole. The fungal development was very satisfactory at this level, and wheat yellow rust developed better than at 100 ppm. At 40 ppm barley leaves became chlorotic very soon (after 14 days) and died soon after this. Consequently the rust development could not reach any significant level. The same lack of preservation was observed with all the cereal seedling leaves when no benzimidazole was used (0 ppm). At 80 ppm benzimidazole, detached leaves of all the cultivars used in the present experiments exhibited the same resistance responses to the rust isolates as potted plants of these cultivars in the glasshouse.

In considering medium structure, 0.7 % water agar proved most suitable and convenient. Lower agar concentrations tended to immerse the leaves too much, producing areas of condensation on the lower surface of the leaves. This made spore collection difficult. The higher

Table 3.2 Rust development on detached host leaves.

EXPERIMENT & TREATMENT	RUST ISOLATES Oat Crown Rust	Barley Brown Rust	Rye Brown Rust	Wheat Brown Rust	Wheat Yellow Rust	Barley Yellow Rust
Benzimidazole						
0 ppm	+	+ *)	+	+	-	- *)
40 ppm	+++	+ *)	+++	+++	++	+ *)
80 ppm	+++	+++	+++	+++	++	+++
100 ppm	+++	+++	+++	+++	+	+++
Support medium						
Agar 0.4 %	+++	+++	+++	+++	++	+++
0.7 %	+++	+++	+++	+++	++	+++
1.0 %	++	+ *)	++	++	++	+ *)
Cotton Wool	++	+ *)	++	++	++	(+) *)
Filter Paper	++	+ *)	++	++	++	(+) *)
Temperature						
10 °C	++	++	++	++	++	+++
14 °C	++	+++	+++	+++	++	+++
22 °C	+++	+++	+++	+++	(+)	+
Light						
8 h	++	++	++	++	++	++
16 h	+++	+++	+++	+++	++	+++
24 h	++ *)	++ *)	++ *)	++ *)	(+) *)	(+) *)

- = no growth
+ = little growth
++ = satisfactory growth
+++ = very good growth
(+) = not consistent
*) = host chlorosis

concentration (1 %) however did not allow enough contact between the leaf surface and the medium to enable the preservation through benzimidazole to take place as effectively as in the lower agar concentrations, and the leaves wilted sooner. The other media (cotton wool and filter paper) proved by far inferior to agar. The tilted agar method was very useful for the bulking up of inoculum, as the spores falling from the leaves were not wetted by agar and could be collected easily.

In the third experiment the optimal temperature was different for the different rust species. Yellow rusts developed best at a temperature of 14 °C, whereas the other leaf rusts preferred higher temperatures. The leaves were best preserved in the low temperature. As yellow rust is the most difficult to keep in detached leaf culture, the temperature for all the following experiments was kept at 14 °C. The light regime applied at 16 h provided sufficient energy for the photosynthesis of the detached leaves. Leaves kept at constant light tended to become chlorotic much more quickly than leaves kept in a day / night regime.

Discussion

Fuchs and Gaertner (1958) desribed the germination of P. graminis tritici on nutrient silica gel containing egg yolk or coconut milk, and found differentiated appressoria and substomatal vesicles together with branching infection hyphae of up to 1.8 mm length but they could not maintain saprophytic growth. In later work (Williams, 1971; Grambow & Mueller, 1978), it was discovered that the formation of appressoria and substomatal vesicles was not essential for the axenic culture of wheat stem rust. Attempts to induce axenic growth of P. coronata and P.

striiformis in the present work were unsuccessful and it seems that the ability to grow axenically depends on the genetic background of an isolate (Bushnell & Stewart, 1971; Green, 1976).

The nuclear condition of axenically cultured rusts would be expected to depend on the nuclear status of the inoculum used. In the present work urediniospores gave rise, to dikaryotic germ tubes and hyphae. However, where the nuclear conditions of axenic cultures has been studied in the literature, isolates were found which were haploid monokaryotic, dikaryotic or diploid uninucleate (Williams, 1971; Williams & Hartley, 1971; Maclean & Scott, 1974; Maclean et al., 1974; Grambow & Mueller, 1978). All these cultures had their origin in urediniospore inoculations. Cultures derived from spermatia are reported to be monokaryotic (Deml et al., 1982a; b). The existence of binucleate basidiospores has been reported previously (Allen, 1933; Wright, 1977; Wright & Lennard, 1978; Anikster, 1983).

With respect to detached leaf infection, Bjoerkmann (1960) found, in his experiments with primary leaves of oats, that the most suitable concentration of benzimidazole was 40 ppm when the leaves floated on the solution and 60 ppm when the petri dishes were tilted and the leaves therefore only partly in contact with the medium. Wolfe and Macer (1964) used a solution of 50 ppm benzimidazole together with 10 ppm kinetin in distilled water to culture wheat and barley yellow rust (P. striiformis). A solution of 40 ppm benzimidazole in water was used for the completion of life cycles of P. hordei and Uromyces scillarum on detached leaves of their main and alternate hosts by Lumbroso et al. (1976). Browning (1954) and Samborski et al. (1958) reported that detached leaves of rust resistant oats or wheat became susceptible when floated on water. Wheat leaves regained their

resistance if 40 ppm benzimidazole was added and 1 % glucose repressed the effect of benzimidazole (Samborski et al., 1958).

Most researchers used a liquid medium for detached leaf culture (Clinton & McCormick, 1924; Waters, 1928; Person et al., 1957; Samborski et al., 1958; Bjoerkmann, 1960; Wang et al., 1961; Wolfe and Macer, 1964; Lumbroso et al., 1977) although some used filter paper (Hennessey & Sackstone, 1970). The agar method in the present experiments was chosen mainly for its convenience and reliability. The most reliable and convenient way of experimenting with cereal leaf rusts in the laboratory was to grow them on detached host leaves, maintained on 80 ppm benzimidazole in 0.7 % water agar; a 16 h daily light regime was found to be most suitable while the temperature of incubation depended on the rust species concerned.

CHAPTER 4

Cytogenetical Studies

Introduction

The urediniospore stage of the rust fungi is characterised by the repeated production of dikaryotic spores in a theoretically unlimited number of generations. Spores which succeed in infecting host plants lead to new spore production after only 7 to 10 days of infection and this cycle can be repeated many times in favourable conditions, possibly leading to vast increases in spore population and severe epidemics on susceptible hosts. Much effort has been invested into the breeding of cereal varieties resistant to the rust diseases. Yet, in many cases, the success of specific resistance genes of a cultivar was shortlived due to the occurrence of new virulences or virulence combinations in the rust population (Van der Plank, 1963; Groth, 1984). Three different possibilities which could lead to such changes in cereal rusts have been discussed in the literature (Zadoks, 1959; Watson, 1970): a) gene mutation (Gassner & Straib, 1932; Johnson & Newton, 1946; Watson, 1957; Zimmer et al., 1963; Macer, 1967; Chamberlain et al., 1970; 1971; Groth, 1984); b) sexual recombination (Craigie, 1927; Allen, 1930; 1931; Green & McKenzie, 1967; Green, 1971); c) somatic recombination (Nelson et al., 1955; Nelson, 1956; Watson & Luig, 1958; Bridgmon, 1959; Ellingboe, 1961; Parmeter et al., 1963; Macer & Doling, 1966; Bartos et al., 1969; Little & Manners, 1969 a & b; Tinline & MacNeill, 1969; Sharma & Prasada, 1970; Hartley & Williams, 1971; Goddard, 1976; Wright, 1977; Wright & Lennard, 1980;

Newton et al., 1981).

The natural rate of mutation for virulence in the rust fungi has been estimated at 10^{-5} by Flor (1958) who worked with Melampsora lini, but this value differs between genes and between the rust organisms (Watson, 1970). Sexual recombination of virulence genes can take place if spermatia can be found in the life cycle of the rust. This stage occurs on the alternate hosts of the cereal rusts. Alternate hosts bearing spermatia and giving rise to aecia have been reported for all cereal rusts apart from P. striiformis. A potential source of variation in virulence in all the cereal leaf rusts is through somatic recombination in dikaryotic hyphae during the uredinial stage: an exchange of nuclei can lead to modified genotypes, while parasexual phenomena have been reported (Bridgmon, 1959; Ellingboe, 1961; Tinline & MacNeill, 1968; Sharma & Prasada, 1970; Watson, 1970). A basis for somatic recombination is the bringing together of different nuclei in the dikaryotic mycelium (heterokaryosis) which may be effected by hyphal anastomosis between different strains of the pathogen (McIntosh & Watson, 1982; Anikster, 1984). The fusion of germ tubes, appressoria and substomatal vesicles has been observed (Allen, 1926; 1928; Bampton & Manners, 1957; Wilcoxon et al., 1958) as well as anastomosis between vegetative hyphae (Wright, 1977; Wright & Lennard, 1980).

Two experiments were carried out in the present studies aimed to clarify cytogenetical properties of the cereal leaf rust fungi.

Materials and Methods

Experiment 1: To observe the formation of hybrids between two different physiologic races of P. striiformis, mixed inoculations were applied onto a universally susceptible host, the mutually resistant

hosts and on a host on which only a somatic recombinant could grow. Wheat yellow rust races 41E136 and 108E9 were inoculated onto detached seedling leaves of the wheat cultivars Heines Kolben, Chinese 166, Norman and Michigan Amber. The respective resistance and virulence combinations and the expected rust reaction are listed in Table 4.1. Two leaves of each cultivar were arranged at random on 80 ppm benzimidazole water agar (0.7 %) in 10 cm square plastic petri dishes. Twenty such petri dishes were inoculated separately with a mixture of approximately equal parts of the two rust races. After inoculation the leaves were incubated at 4 °C for 24 hours in the dark and then for a total of 21 days at 14 °C in a 16 hour light 8 hour darkness regime. A qualitative assessment of infection was done after 21 days. Where spores developed from mixed infections after this time, they were reinoculated onto the whole set of cultivars and sporulation was assessed after another 21 days. The experiment was carried out on two different occasions.

Table 4.1

Resistance and virulence factors of four wheat cultivars and two wheat yellow rust isolates and expected infection.

	Cultivar (resistance factor)			
	Heines Kolben (RF 6)	Heines VII (RF 2)	Norman (RF 2 & 6)	Michigan Amber (RF 0)
Isolate (Virulence)				
108E9 (VF 3,4 & 6)	+	-	-	+
41E136 (VF 1,2 & 3)	-	+	-	+

+ compatible - incompatible

Experiment 2: Urediniospores of wheat and barley brown and yellow rusts were germinated on host leaves and on water agar at 14 °C. After

24 hours samples were taken, stained with the nuclear fluorescent dye mithramycin (see Appendix 4.1) and observed under the fluorescence microscope. The position and number of nuclei in the germ tubes and the appressoria of these fungi was examined as well as any fusions between germ tubes, appressoria or sub-stomatal vesicles. Over 1000 germinations were examined.

Results

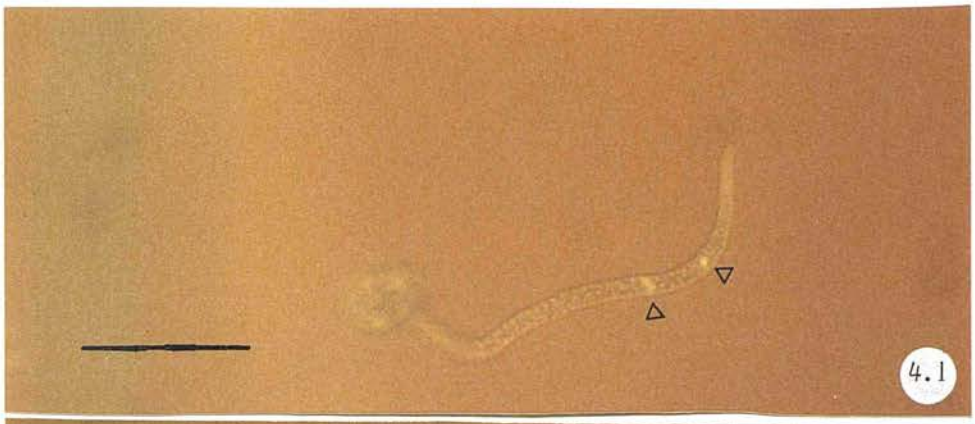
Experiment 1: Single inoculations of the two isolates produced the expected infections listed in Tab.4.1. Mixed inoculations led to infections and sporulation on cvs. Michigan Amber (RF 0), Heines VII (RF 2) and Heines Kolben (RF 6), but no sporulation could be scored on cv. Norman (RF 2 & 6). Reinoculation of spores from the three compatible combinations onto the whole set of cultivars showed no evidence of recombination of the virulence factors VF 2 and 6 between the two isolates in this experiment. Only parental types could be recovered. The repetition of the experiment led to the same result.

Experiment 2: The number of nuclei throughout the experiment was two. No nuclear divisions could be observed up until the formation of substomatal vesicles and the stain used in this experiment did not reveal the fungal nuclei beyond this stage. The position of the nuclei in the germ tubes of P. triticina and P. striiformis were not well defined. One nucleus was positioned between 30 and 90 µm behind the germ tube tip and the other nucleus was following at a distance of 17 to 70 µm (Plates 4.1 and 4.2). In P. hordei germ tubes the nuclei were always in close association with each other 12 to 17 µm apart and positioned approximately 40 µm behind the germ tube tip, but not closer to it than the last germ tube branch (Plates 4.3 and 4.4). The sizes of

Plates 4.1 - 4.4 Dikaryotic urediniospore germ tubes of P. triticina and P. hordei.

Plates 4.1 & 4.2 P. triticina; nuclei (◁);
FM; mithramycin stain;
length of bar = 50 µm.

Plates 4.3 & 4.4 P. hordei; nuclei (◁);
FM; mithramycin stain;
length of bar = 10 µm.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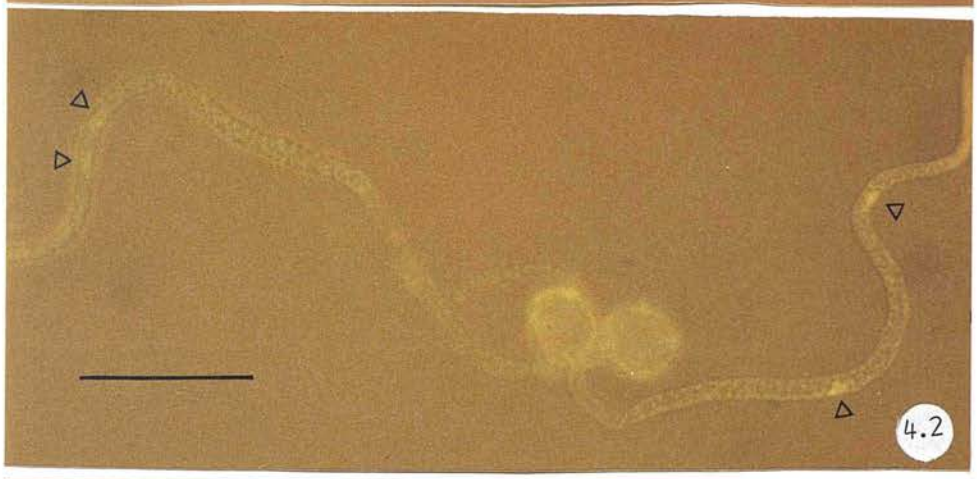
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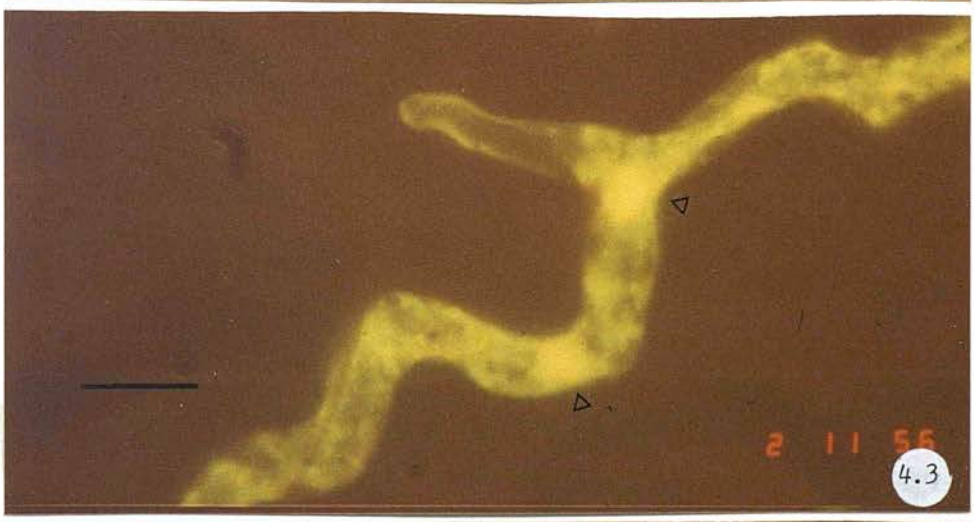
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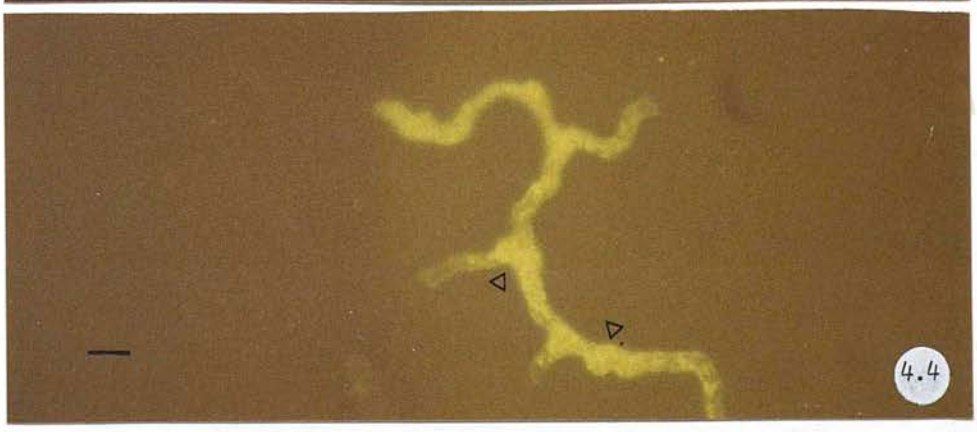

4.4

the nuclei were very similar for the three species examined in this experiment. They measured approximately 5.9 X 3.5 µm but the assessment was made difficult by the instability of the fluorescent dye, which faded after only about 30 sec. of incident ultraviolet light. No fusion of germ tubes, appressoria or sub-stomatal vesicles could be recorded. Although in many cases two or three appressoria occupied the same stoma, the fungi always entered the host separately forming separate sub-stomatal vesicles and infection hyphae.

Discussion

A number of workers have reported somatic recombination of virulence genes in P. striiformis (Macer, 1967; Little & Manners, 1969a; Goddard, 1976; Wright, 1976; 1977; Wright & Lennard, 1980; Newton et al., 1981). In all reported cases a simple exchange of nuclei between two races was possibly the cause of the recombination, as more complex rearrangements of genes which would have been due to parasexual phenomena could not be detected. Some characters, such as the spore colour might also be inherited in the cytoplasm (Newton et al., 1981). Bridgmon (1959) and Ellingboe (1961), and later other workers, reported on parasexual phenomena in P. graminis and other cereal rusts, whereas Flor (1964) could explain all the variation in Melampsora lini by mutation and the exchange of whole nuclei, without any evidence of parasexuality.

In 1926 Ruth Allen observed fusion phenomena of appressoria and sub-stomatal vesicles in P. triticina, but not in P. striiformis (Allen, 1928). Later, germ tube fusion was discovered in P. graminis (Rodenhiser & Hurd-Karrer, 1947; Bampton & Manners, 1957; Wilcoxson, 1958) and P. striiformis (Little & Manners, 1969b). Work by Nelson

(1956) and Bridgmon (1959), as well as more recent work (Macer, 1967; Goddard, 1976; Wright, 1976; 1977; Wright & Lennard, 1980) indicated fusion of vegetative mycelium (hyphal anastomosis) as a basis for genetical recombination, and Wright (1977) showed micrographs which could represent fusion structures. In the present experiments (Experiment 1) no recombination of virulence genes could be obtained. A race combining the virulence factors VF 1,2,3,4,6 & 7 has been described in the literature from experimental inoculations (race P631; Bayles & Thomas, 1984; 1985), and other races combining VF 2 and 6, and virulent on cultivar Norman, have been isolated in nature (Priestley et al., 1982; Bayles & Priestley, 1983; Bayles & Thomas, 1984; 1985). As no fusions of sub-stomatal vesicels have been reported for P. striiformis in the literature and also none could be found in these experiments (Experiment 2), the most likely mechanism of recombination is the anastomosis of intercellular hyphae. This may be the reason why somatic recombination on a host, resistant to one or both isolates in a mixture is not likely to occur, as the mycelial development in such a host and therefore the chance for hyphal anastomosis is impeded. On the other hand recombination of virulence genes could have taken place on the susceptible cultivar Michigan Amber (RF 0), and other workers have obtained relatively high rates of somatic recombination on a universally susceptible host working with different yellow rust races (Little & Manners, 1969a; b; Goddard, 1976). However, Wright (1977) reported no recombination on the universally susceptible cultivar Sappo, and Watson (1957; 1981) showed that the mixture of some races of P. graminis did lead to recombinants, whilst others did not.

In Experiment 2 the number of nuclei in urediniospores, germ tubes and appressoria of the three rusts under investigation was always

two. Allen (1926) found two nuclei in the spores, four in the appressoria and eight in the sub-stomatal vesicles of P. triticina, and nuclear division phenomena during appressorium formation have been described by other workers (Wolf, 1982; Staples & Macko, 1984). The failure of recording more than two nuclei in the present experiments might be due to the close association of daughter nuclei which might appear as the same body when the fluorescent dye mithramycin is used. The relatively big size of the nuclei for P. striiformis and P. hordei could be another indication of this. In her experiments with P. triticina, Allen (1926) found fusion phenomena of either appressoria or sub-stomatal vesicles in 4 % of the sporelings, and for P. graminis the fusion of germ tubes has been reported (Bampton & Manners, 1957; Wilcoxson, 1958), but no confirmation of these findings was made in later work on the hybridisation of rust genes. Germ tubes often crossed each other in the present experiment and on one SEM micrograph seemed to fuse (Plate 4.5). Yet, closer investigation showed that only their outer layers, possibly a mucilaginous exudate, became united and the tubes separated again later and penetrated the leaf with two separate appressoria (Plate 4.6).

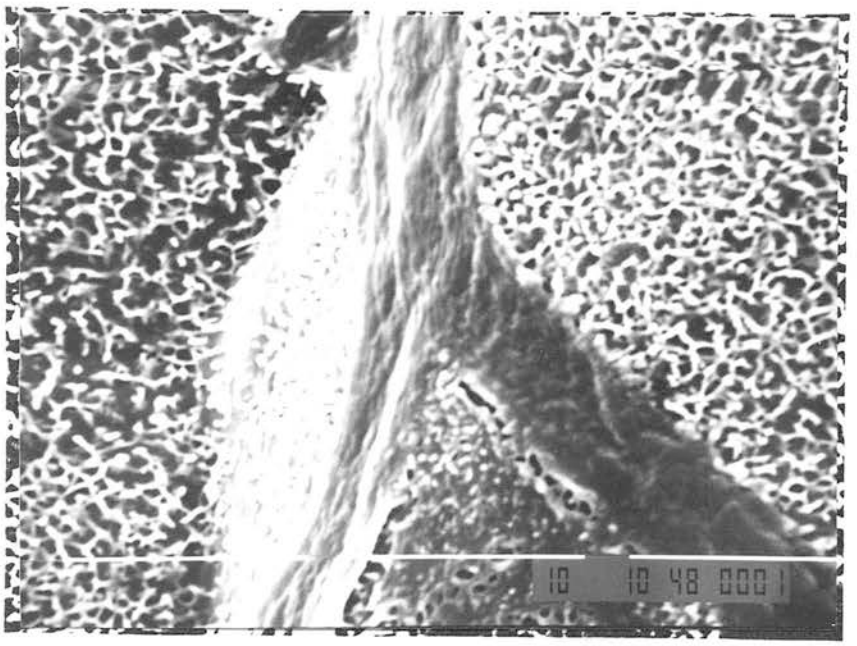


Plate 4.5 Apparently fusing germ tubes of P. triticina;

SEM;

length of bar = 10 µm.

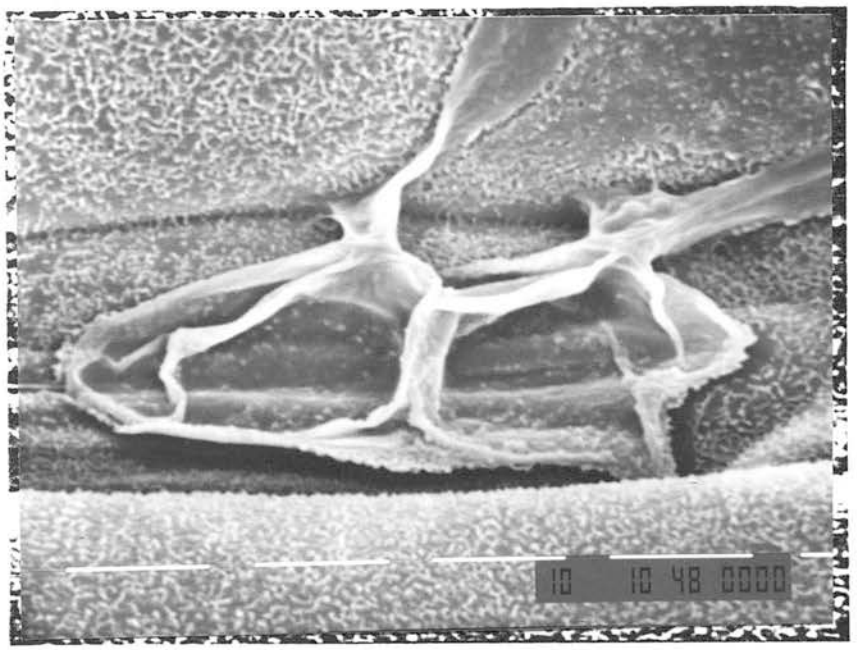


Plate 4.6 Stomatal penetration via two appressoria;

SEM;

length of bar = 10 µm.

CHAPTER 5

Physiological studies

General introduction

The growth and development of cereal leaf rust fungi and their spread and epidemic potential within a crop are determined by a number of factors (Zadoks, 1972; Loegering, 1984). Some of these factors are inherent genetical properties of the pathogen, others are dependent on the physiological condition of the fungal cells and others again are external factors and relate to the susceptibility and predisposition of the host plant or to the chemical, physical or biological factors of the environment. The spread of disease in a particular crop is thus dependent on the genetic compatibility between pathogen and host, the physiological condition of the pathogen, the condition of the host plants and also on the weather and biological factors which influence the spread of the pathogen within the crop.

The present chapter describes experiments, considered in three parts, aimed to elucidate the genetical and physiological attributes of rust fungi and host plant relationships. Firstly, factors affecting the germination of urediniospores are considered. A second part describes the development of P. hordei and P. striiformis on barley in relation to different inoculum densities, and examines the factors influencing sporulation when successful colonisation has taken place. Finally, experiments on growth and development of rust isolates on host and non-host cereal plants are presented.

A) Germination of urediniospores.

Introduction

The first stage of rust development and an important step in the induction of infection is spore germination: this subject has created much interest, and many different aspects of urediniospore germination have been identified and described in the literature. Among these aspects are the reaction of spores to different physical and chemical factors in the environment at the time of germination, as well as to internal factors of the spore derived from influences present during the spore formation and subsequent storage or other treatments. In 1923, Allen described the germination of P. graminis on susceptible and resistant wheat plants. Stock (1941) published his work on germination and germ tube elongation of several other rusts. Later, physiological studies were reported on the germination of urediniospores of P. striiformis (Straib, 1940; Schroeder & Hassebrauk, 1964; Sharp, 1965; Strobel, 1965; Tollenaar & Houston, 1966; Russell, 1976; Cartwright & Russell, 1981), P. graminis (Fuchs & Gaertner, 1958; Gaertner & Fuchs, 1962; Tollenaar & Houston, 1966; Knights & Lukas, 1981), P. triticina (Clifford & Harris, 1981), P. hordei (Niks, 1982), and other rusts (Maheshwari et al., 1967). Investigations into the biochemistry of germinating spores were carried out by many workers in more recent years and reviews of their findings have been published (Shaw, 1963; 1964; Allen, 1965; Staples & Wynn, 1965; Staples & Yanif, 1976; Wynn & Staples, 1981; Wolf, 1982; Staples & Macko, 1984). Of particular interest is the occurrence of self inhibitors and stimulators of germination, first discovered by Allen (1955) and later isolated and

purified (Wilson, 1958; Bell & Daly, 1962; Staples & Macko, 1984).

The present experiments were carried out to provide a better understanding of the germination patterns of urediniospores and of the factors influencing germination rate and efficiency: the results were of interest for the spore density experiments (this Chapter, Section B) and for the assessment of epidemic potentials of particular isolates.

Materials and Methods

A series of experiments was carried out on germination of different rust fungi to study variation among different species and isolates within species, and the effect of substrate and temperature on germination.

Urediniospores of the cereal leaf rust fungi were taken from the universally susceptible cereal cultivars for each rust species (see Appendix 1.1) grown at 14 $\pm$ 2 °C. The spores were gently shaken off the leaves and collected in a glass tube. They were then subjected to various treatments or used directly for inoculation of host leaves or agar surfaces. The inoculation was carried out in two different ways. Spores were suspended in 0.05 % Tween 20 (polyoxyethylenesorbitane monolaurate) in sterile distilled water or applied directly, as spore powder. In both cases application was carried out, using a Humbrol spray gun and freon (CCl_2F_2) as carrier gas to provide a fine spray of suspended spores or a cloud of dry spores. The nozzle of the spray gun was directed vertically into a spore settling tower, and the spores were allowed to settle onto the respective surfaces in the lower part of the tower (see Plates 5.1 & 5.2 and Fig. 5.1). Water agar (0.7 %) and detached cereal leaves on 80 ppm benzimidazole water agar were used as surfaces for spore germination.

Plate 5.1 General view of spore settling tower

Plate 5.2 Spray gun used for inoculation; Pasteur pipette containing inoculum (◀).

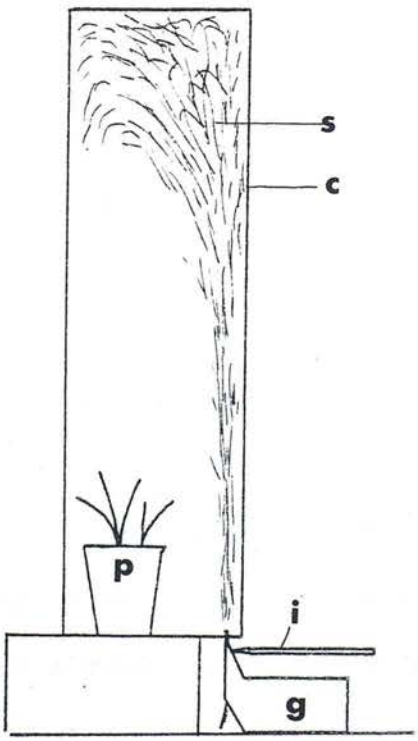


Fig. 5.1 Diagram of spore settling tower;
c aluminium cylinder
g aerosol gun
i pasteur pipette containing inoculum
p plant material
s spore cloud.

The spores were incubated at 4 °C in the dark or at 14 ± 2 °C in a 16 h light / 8 h darkness regime. On the agar surfaces germination was assessed after various times of incubation using a Kyowa stereo-microscope with dark field condenser, and counting all the spores as germinated which had developed germ tubes longer than the spore's diameter. Germination on the cereal leaves was assessed after 24 hours using SEM and fluorescence microscope techniques (see Appendices 2.2 & 2.3).

Experiment 1 was carried out with two isolates of P. hordei at two different temperatures. Isolates BBR 76-12 and BBR race A were multiplied on whole plants of the universally susceptible barley cultivar Midas at 14 ± 2 °C, and urediniospores were harvested 14 days after inoculation. Spores were suspended in 0.05% Tween 20 and sprayed onto 0.7% water agar in 90 mm diameter plastic petri dishes. The petri dishes were incubated for 24 hours at 15 and 20 °C in the dark in growth cabinets. After this period the germination rate was assessed. The experiment was carried out using four replicates. Experiment 2 assessed germination of different isolates of P. triticina on water agar, Experiment 3 compared P. striiformis (BYR) and P. hordei on barley seedling leaves, while Experiment 4 investigated germination of P. coronata and 12 isolates of P. striiformis (WYR) on water agar. A fifth experiment was carried out using an isolate of P. striiformis (BYR BYV 1) on barley, to assess whether the germination rate on water agar was influenced by the presence of healthy or infected host leaves. Seedling leaves of barley cv. Berac (fully susceptible) were inoculated with spores of P. striiformis suspended in 0.05 % Tween 20. Control leaves were inoculated with the Tween 20 solution only. Both treatments were incubated at 14 ± 2 °C in the greenhouse after an initial

incubation at 4 °C for 24 hours. Three days later the leaves were detached and placed on water agar on which fresh urediniospores of the same rust isolate had been previously sprayed. The plates were incubated for two days at 4 °C and germination was assessed on the agar in an area within 1 mm from the detached leaves. The germination on agar alone, without the influence of leaves was also measured. The experiment was replicated four times.

Results

The results of the spore germination experiments are presented in Table 5.1.

Fresh spores germinated generally well and at high percentages in favourable conditions (14 °C, high relative humidity) on water agar as well as on cereal leaves. Fresh spores started to germinate after 30 min on water agar. The first sign of germination was a swelling of the germ pores (Plate 5.3). One or occasionally two germ tubes began to extrude from the spore after about 60 min (Plate 5.4), but only one germ tube continued to grow (Plates 5.5 to 5.9 and see also Plate 2.17). Some isolates (of P. striiformis, P. triticina and P. coronata) produced unbranched germ tubes which, on water agar, grew for a considerable length; others developed germ tubes with short branches (P. hordei) or even branched dichotomously as with P. recondita (see also Chapter 2). On host plants germ tubes grew across the leaves at right angles to the leaf veins. It was observed that, in establishing inoculum after vacuum drying and storage under refrigeration, urediniospores of all isolates of the cereal leaf rust fungi examined in this experiment germinated at very low rates (under 0.1 %), yet infection of host plants could still be achieved after 30 months of

Plates 5.3 - 5.9 Germination.

Plate 5.3 Swollen germ pore of P. hordei; shortly after inoculation; SEM;
length of bar = 1 µm.

Plates 5.4 - 5.8 Urediniospores of P. hordei on water agar (1 %); LM;
X 180.

Plate 5.4 at 60 min. after inoculation;

Plate 5.5 at 120 min. after inoculation;

Plate 5.6 at 150 min. after inoculation;

Plate 5.7 at 180 min. after inoculation;

Plate 5.8 at 210 min. after inoculation;

Plate 5.9 Urediniospores of P. striiformis on water agar (1 %); at 150 min. after inoculation;
LM;
X 180.

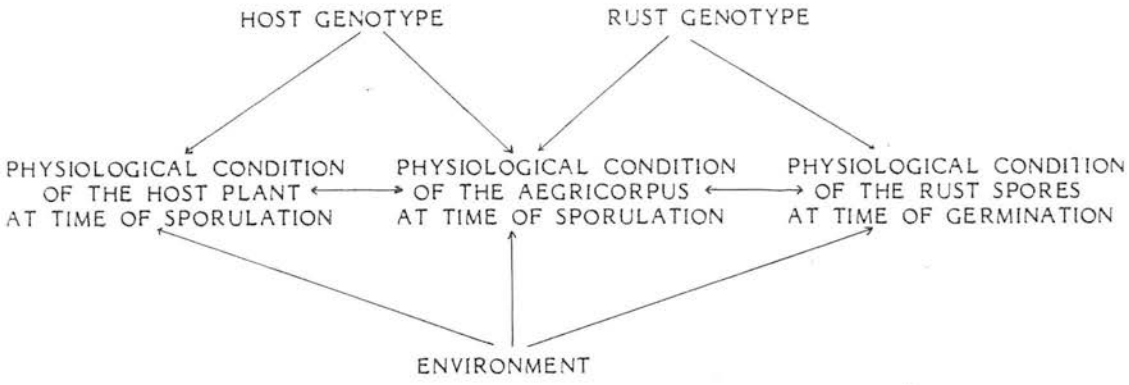
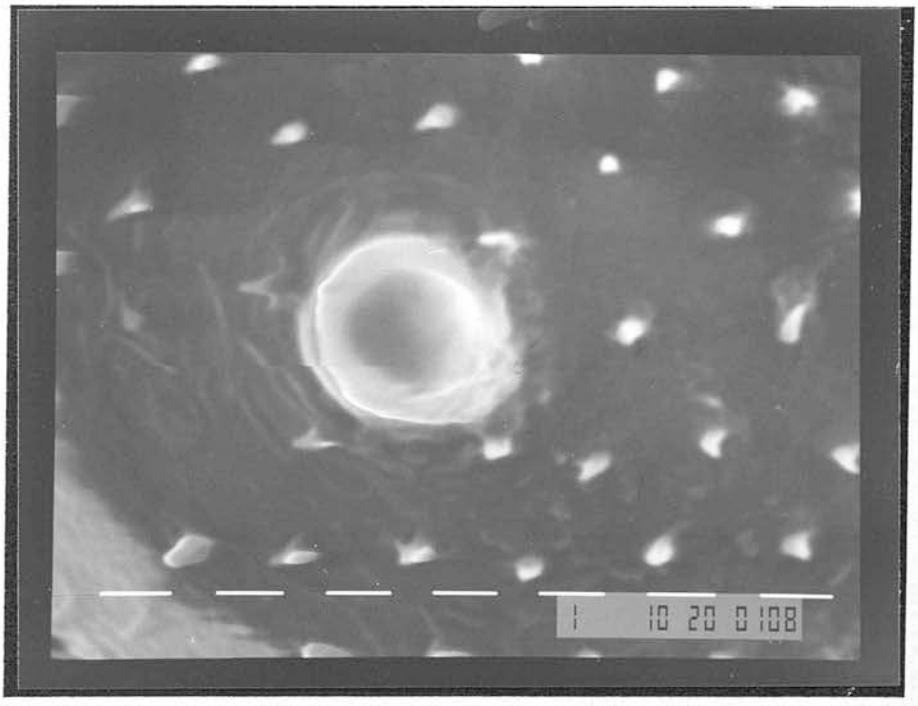
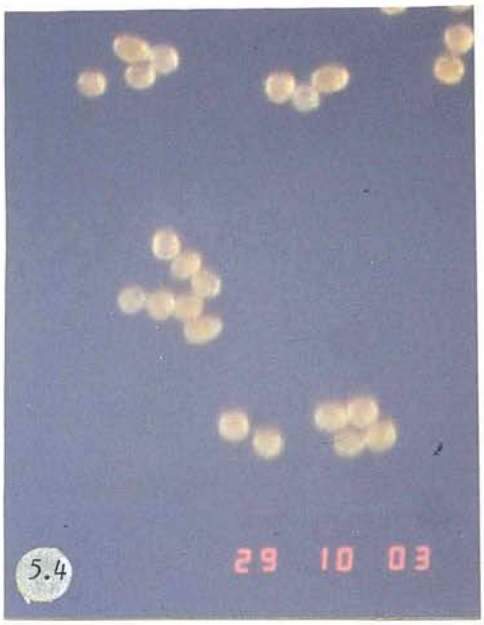


Fig. 5.2
Factors influencing spore germination

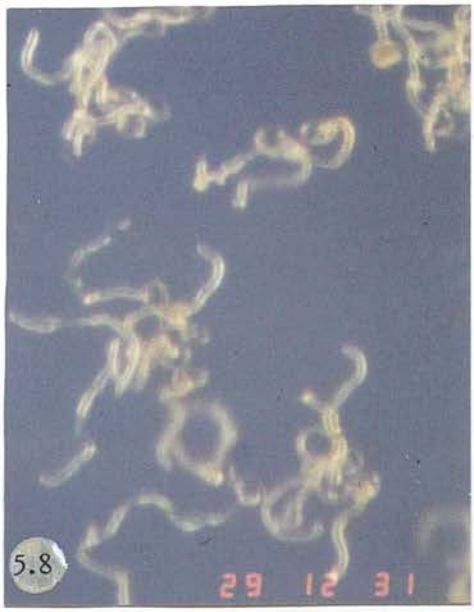



5.4
29 10 03


5.5
29 11 00


5.6
29 11 31


5.7
29 12 01


5.8
29 12 31


5.9

Table 5.1
Results of germination experiments with rust urediniospores.

Experiment	Isolates & Methods	Germination rate %		SED ±	Range of germination % rates between replicates
1		15 °C	20 °C		
	BBR race A	76.5	75.4	5.53	70 - 88
	BBR 76-12 (in Tween 20)	34.5	31.7	4.20*	19 - 46
2	WBR 74-2	59.7			30 - 91
	77-22	71.3			30 - 97
	79-4	76.3			51 - 92
	79-21	66.7			15 - 95
	(on water agar 0.7%; at 14 °C)			10.24	
3	BYR 1	6.9			3 - 15
	BBR 83-2	69.1			64 - 71
	(on barley seedlings at 14 °C)			3.38	
4	OCR	77.0			65 - 89
	WYR P631	79.5			75 - 84
	71-493	85.5			76 - 95
	72-23	44.5			35 - 54
	75-27	9.5			4 - 15
	76-15	25.6			16 - 35
	80-21	17.6			8 - 27
	81-11	54.0			38 - 70
	37E132	52.4			43 - 62
	41E136	21.5			12 - 31
	104E137	69.5			60 - 79
	104E137W	10.5			1 - 20
	108E9	78.5			69 - 88
	(on water agar 0.7%; at 14 °C)			6.69	
5	BYR 1	uninoculated leaves	inoculated leaves		
	agar surrounding leaves	65.2	6.8	1.83	4 - 78
	uninfluenced water agar	59.9	56.5	1.43**	54 - 67
	(on water agar 0.7% with uninocolated and previously inoculated leaves, 3 days after inoculation, at 4 °C)				

* when compared with same levels of temperature.
** when compared with same levels of leaf inoculation treatment.

storage when high spore densities were applied.

In Experiment 1 highly significant differences in germination rate could be discovered between the spores of different isolates of P. hordei but no significant difference between the two temperatures, nor any interaction of temperature and isolates could be established in

this experiment. With P. triticina no significant difference in spore germination between four isolates was recorded (Experiment 2). In Experiment 3, P. striiformis showed a significantly lower germination rate than P. hordei. The isolates of P. striiformis examined in Experiment 4 varied greatly in their germination rate. In Experiment 5, germination on the agar surrounding the previously inoculated leaves was substantially inhibited, compared with spore germination on unaffected agar. A slightly higher percentage of spores germinated in the proximity of healthy leaves than on uninfluenced agar and this difference was significant. The nature of the inhibition and stimulation of germinaton was not determined.

Discussion

The results presented in Table 5.1 suggest that the urediniospore germination is a very variable feature in the cereal rust fungi. This variability must be recognised in experiments, as even small differences in conditions prior to inoculation can influence the germination rate considerably. In experiment 1 the two isolates showed significant differences in germination rate, having both been raised in apparently optimal conditions. Yet, it is difficult to imagine that inherent genetical factors should be present which inhibited some of the spores from germinating, where others germinated without inhibition. In a genetically homogenous spore population all spores in a given physiological state should be reacting similarly (all isolates in use throughout these experiments were derived from single snore isolations of the given races, provided by different institutes and from departmental collections; c.f. Appendix 1.2). It is therefore likely that spores which failed to germinate were disadvantaged physio-

logically. Isolate BBR 76-12 apparently suffered a disadvantage due to factors determined by the way the experiment was carried out. Reports in the literature indicate that differential physiological requirements of certain isolates of a given rust species can lead to a dominance of these isolates over others under specific conditions (Falahati-Rastegar et al., 1981; 1983). This could explain in part why the distribution of rust races is not uniform worldwide. The physiological requirements for spore germination may be determined by genetical factors or by environmental conditions during the time of spore production (Straib, 1939; Schroeder & Hassebrauk, 1964; Hassebrauk, 1970). It is therefore difficult to assess the source of variation in spore germination. The rust genotype plays obviously a major role, as does the environment (Fig. 5.2); if the environmental conditions are kept uniform, variation can still occur due to a number of interactions between host and pathogen.

The results presented in Experiments 2 to 4 again suggest the variable nature of spore germination of other rusts due to the complexity of the factors involved.

In Experiment 5 an inhibition of germination with previously inoculated leaves was evidenced. Its origin could be from the developing rust mycelium in the infected barley leaves or from any interaction between host and parasite. Allen (1976) reviewed a number of papers concerning the inhibition of spore germination originating from the parent colonies, yet none of these deal with rust fungi. In experiments with P. triticina, Bahamish & Wood (1985) inoculated different wheat plants with virulent or avirulent isolates of wheat brown rust in successive inoculations after several time intervals. They reported very significant differences in the production of urediniosori in

leaves inoculated twice compared with leaves inoculated only once, yet no reference is made to an influence of developing colonies on the germination of spores of the second inoculation.

B Infection responses to different levels of inoculum density in P. hordei and P. striiformis on barley.

Introduction

One of the factors influencing the outcome of infection is the density of the inoculum deposited on the host plant. This is particularly true for diseases which, like the cereal brown rusts or mildews, cause only a limited infection on their hosts, and only partly true for diseases, like the ones caused by Ustilaginales and some members of the Uredinales (Endophyllum sempervivi, Uromyces pisi on Euphorbia cyparissias or Transchelia fusca), causing systemic infection. P. striiformis, with its semi-systemic habit lies in between these two extremes. Peterson (1959) first studied the infection response of wheat to inoculum densities of the wheat stem rust fungus Puccinia graminis. Later a similar study was carried out with the bean rust Uromyces phaseoli (Davison & Vaughan, 1964), where different infection types could be linked to the infection density applied to the leaves. Together with the spore germination and germ tube penetration efficiency, as well as various agencies of dispersal (see Van der Plank, 1975), the number of propagules from a single infection is a very important measure of the epidemic potential of a parasite on a given host. This measure estimates the success of the pathogen more precisely than the classic disease rating does and is therefore a more meaningful assessment of resistance response and disease development

(Zadoks, 1972; Helfer, 1985).

The present studies were aimed to assess the infection efficiency of P. hordei and P. striiformis in relation to their inoculum density. Particular emphasis was laid on the spore production and the multiplication rate of the fungus.

Materials and Methods

The experiments were carried out on detached leaves of fully susceptible barley cultivars maintained on water agar containing 80 ppm benzimidazole, in 100 mm square petri dishes. Different spore loads of P. striiformis and P. hordei were inoculated onto leaves using spore suspensions in 0.05 % Tween 20. The inoculations were carried out using the same procedures as in part A, this chapter. After inoculation the leaves were treated separately, as replicates, and incubated at 4 °C for the initial 16 h, followed by the final incubation conditions at 18/14 °C in a 12 h light, 12 h darkness regime in growth cabinets for the rest of the time of the experiment. At 24 h after inoculation samples were taken from the leaf segments and prepared for SEM observations (see Appendix 2.3). Spore density, germination rate and germ tube pene- tration rate were assessed using the scanning electron microscope. Spore density and germination rate were also determined on the agar using the light microscope.

After 10 days, the first brown rust urediniosori erupted. The numbers of uredinia were counted and the leaf area between two boundary lines, 50 mm apart, was measured. Spores produced in these sori were collected daily from day 10 onwards (apart from days 13 and 14) and the spore number was determined. Yellow rust sori emerged from day 13 onwards and spores were collected on days 15-19. As the number of yellow rust sori increased rapidly with the development of the fungus

Table 5.2

Inoculum densities (germinating spores/cm^2) used in experiment B.

	very high	high	medium	low
Isolate BBR 83-2	888	125	68	10
Isolate BYR race 1	-	181	51	21

during the experiment, no spore-sorus ratio could be established. The spore collection was carried out using a suction method (Helfer, 1985; Appendix 5.1) which left the leaves completely undisturbed. The experiment was carried out with five replicates.

Results

The spore counts of three inoculum densities are plotted against the date of collection in Fig. 5.3 for barley brown rust and Fig. 5.4 for barley yellow rust. In Fig. 5.3 the very high inoculum density is added to the graph of high, medium and low densities.

Urediniospore germination percentages assessed on the leaf surfaces were 76.6 $\pm$ 4.8 for BBR 83-2 and 6.78 $\pm$ 6.70 for BYR race 1. The densities of germinating spores per cm^2 are shown in Table 5.2. Of these germinating spores, only 49.3 $\pm$ 9.7 % for BBR and 28.8 $\pm$ 4.5 % for barley yellow rust penetrated their host leaves.

The formation of urediniosori in brown rust was at a rate of 14.5 $\pm$ 3.06 % of germinating spores (36.8 $\pm$ 10.1 % of penetrating germ tubes). From day 20 onwards the infections from low and medium inoculum densities produced secondary sori around the exhausted initial uredinium (see also Plate 2.23, Chapter 2). In brown rust the sporulation per square centimetre leaf surface was highest at the high

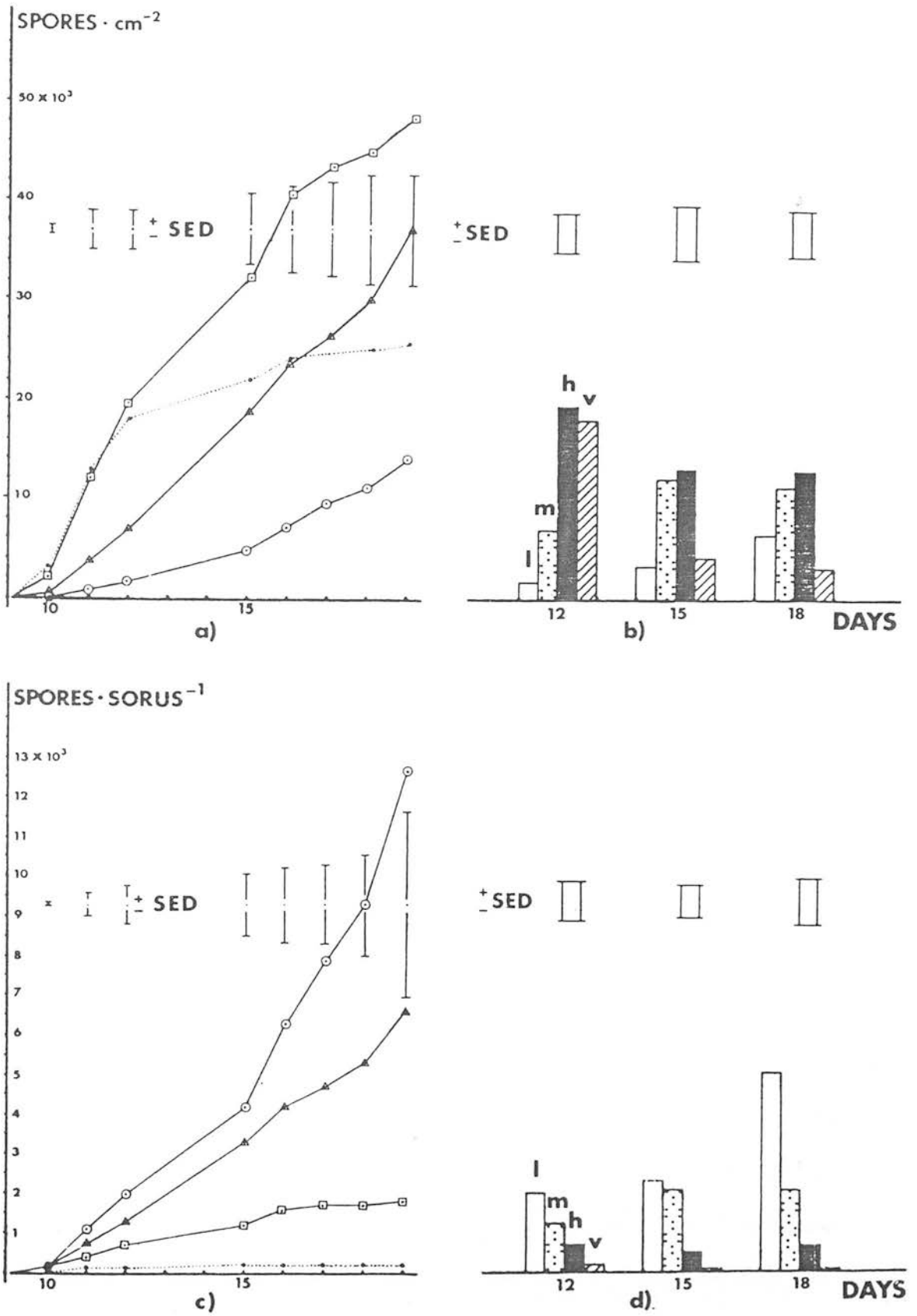


Fig. 5.3
Spore production on barley of P. hordei at low (l; ⊙—⊙), medium (m; ▲—▲), high (h; ⊡—⊡) and very high (v; ·····) inoculum densities; a) & b) show spore production per cm2; c) & d) show spore production per sorus.

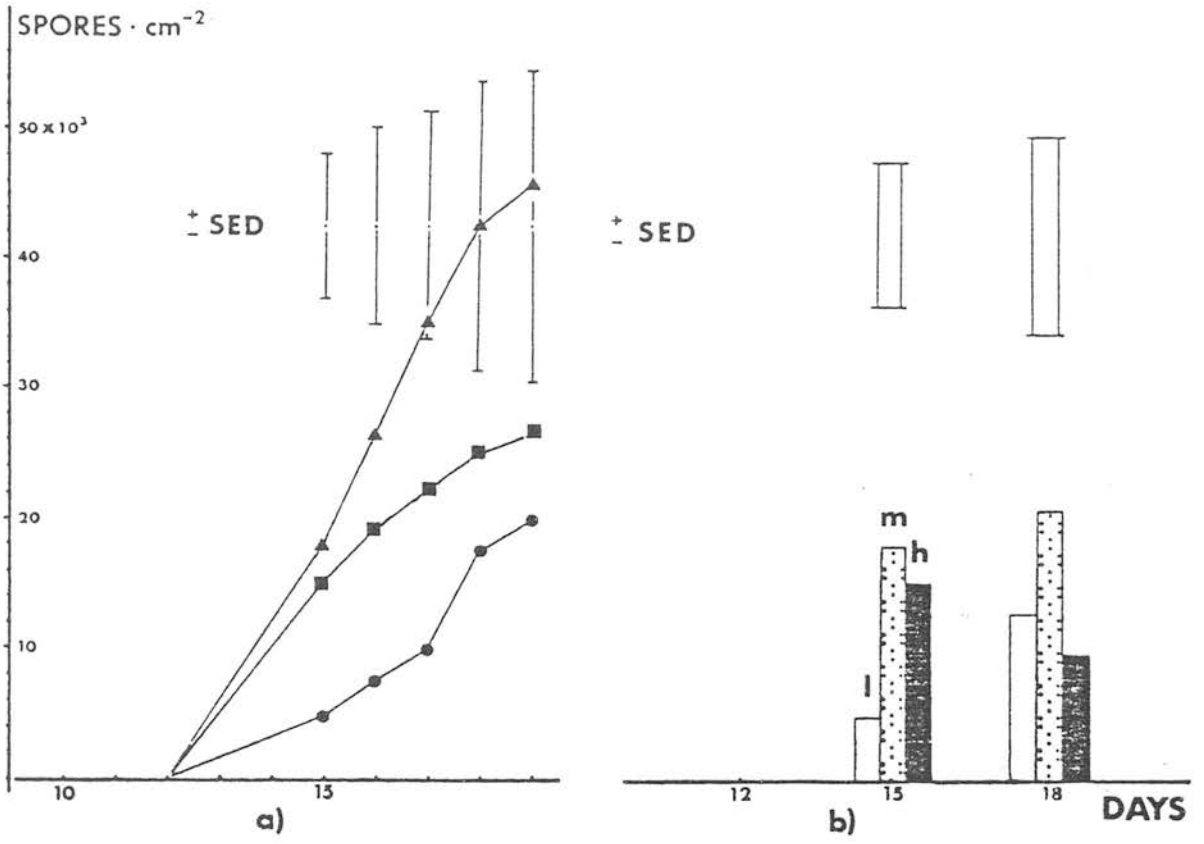


Fig. 5.4
Spore production on barley of P. striiformis at low (l; ●——●), medium (m; ▲——▲) and high (h; ■——■) inoculum densities.

inoculum density (Fig. 5.3 a; 125 spores/cm²) from day 12 onwards, whereas the very high inoculum (888 spores/cm²) led to higher spore production only on the first 2 days of collection and then rapidly fell behind the yield from the high and even the medium inoculum. From day 12 to 15 the differences in spore yield between high and medium inoculum densities were not significant (Fig. 5.3 b) but the very high and low densities were significantly lower in spore production. In the period from day 15 to 18 the situation tended to be similar, with the low density increasing production slightly and the very high density decreasing even more compared with the previous period. Differences in the cumulative spore production between inoculum densities were highly significant throughout the experiment. When considering the spore production per

Table 5.3
Infection efficiency of barley leaf rusts.

a) Spore production per viable spore of inoculum.

Density Isolate	very high	high	medium	low	SED
BBR 83-2	29	390	547	1390	± 246.6
BYR race 1	-	148	903	1006	± 309.6

b) Sorus production per viable spore of inoculum.

Isolate					
BBR 83-2	14.0	21.8	8.2	14.1	± 3.06
BYR race 1	not applicable				

sorus (Fig. 5.3 c & d) it was found that the fewer sori per surface area produced relatively more spores than when the sori were numerous and in close proximity to each other. It was, however interesting to note that on the first day of collection sori produced roughly the same number of spores irrespective of the sorus density. On all later dates significant differences could be established.

In barley yellow rust the highest total spore production occurred at a medium density of 51 spores/cm^2 (Fig. 5.4 a), compared with the high (181 spores/cm^2) and the low (21 spores/cm^2) densities. The difference in spore production between the densities, however, was never significant.

Table 5.3 shows the infection efficiency of the two barley rusts at 19 days after inoculation. Although the barley yellow rust isolate started sporulation only three days after the brown rust isolate, the total spore production of these two isolates at day 19 over the three comparable inoculum densities was nearly the same and at medium spore loads P. striiformis was more efficient than P. hordei (Table 5.3 a).

Discussion

The germination rate of P. hordei and P. striiformis differed considerably in this experiment. Also, the germination rate of the yellow rust isolate was not very consistent, ranging from 3 to 14 %. As the germination rate of cereal rust urediniospores is dependant on the density of inoculum (Allen, 1955; Petersen, 1959; Falahati-Rastegar et al., 1981; 1983) part of this inconsistency in the yellow rust isolate could be attributed to a crowding effect, but this could not be proved statistically. Petersen (1959) describes this effect at densities exeeding 5000 spores/cm^2 for P. graminis and Falahati-Rastegar et al. (1983) mention the density of 5000 spores/cm^2 as the optimal spore load with highest germination rates. In this experiment no correlation between inoculum density and spore germination could be established as the spore loads employed were well under this number (see also part A, this Chapter). The penetration rate was also very different between the two isolates (28.8 % in yellow rust and 49.3 % in brown rust), but not significantly different between the various inoculum densities. One of the reasons for the lower penetration efficiency of yellow rust could be the absence of appressoria in this fungus: all brown rust appressoria observed in this experiment led to penetration, resulting in the formation of substomatal vesicles and the collapse of appressoria. Petersen (1959) found penetration rates of 28 - 52 % for P. graminis and Stubbs & Plotnikova (1972) rates of 27.2 to 46.7 % for wheat yellow rust on susceptible and 5.1 to 17.6 % on resistant wheat cultivars. In their experiments the spore germination also showed variation between cultivars. Other workers reported various penetration efficiencies for P. striiformis (10 - 20 %; Cartright & Russell, 1981), P. triticina (40 - 70 %; Poyntz & Hyde, 1985; 42 - 58 %; Bahamish &

Wood, 1985) and P. hordei (35 - 52 %; Aslam & Schwarzbach, 1980). Puccinia hordei is also reported (Falahati-Rastegar et al., 1983) to penetrate different host leaves at a rate of 19 - 40 % (of germinating spores), depending on race, cultivar and conditions of incubation at inoculum densities of about 3500 germinating spores/cm^2 For the outcome of infection, Aslam & Schwarzbach (1980) reported in P. hordei a ratio of 10 pustules per 100 applied spores or, in their case, of 44 pustules per 100 germinating spores at inoculum densities of around 100 spores/cm^2 Petersen (1959) achieved far lower efficiencies with P. graminis at an average of 1 urediniosorus per 100 germinating spores and a maximum of 40 sori/cm^2 irrespective of higher inoculum densities. In studies with the bean rust fungus Uromyces phaseoli, Davidson & Vaughan (1964) discovered an optimal sorus density for this parasite on bean cultivars of 30.4 sori/cm^2 with inoculum densities of 1800 - 2000 spores/cm^2. Increasing the spore load resulted in decreasing sorus densities in their experiment. In the present study with P. hordei a ratio of 16.4 sori per 100 germinating spores could be established and the highest sorus density was 124.7 sori/cm^2 at a spore load of 888 spores/cm^2, and no correlation between sorus/spore ratio and inoculum density could be established.

C) Rust development on host and non-host plants.

Introduction:

Being obligate parasites, the cereal rusts are considered to be among the most specialised of organisms. Not only are they confined to a narrow range of main and alternate hosts but, within their main hosts, a particular isolate will develop only on a selection of cultivars, relating to the respective virulence and resistance factors which are present. This relationship was first described for flax and its rust Melampsora lini by Flor (1942). Since then many such gene for gene relationships have also been described for cereal rusts (Roelfs, 1984). The methods currently used for race identification consider the outcome of infection, in a system of various classes of resistance or susceptibility (Hoerner, 1919, McNeal et al., 1971). Although much work has been carried out on rust infection in susceptible responses, for example Pole-Evans (1907) and Rowell (1984), little research has been carried out to investigate the development of rusts on non-host cereals. Ogle and Brown (1971) describe the growth and development of wheat stem rust Puccinia graminis tritici during the first five days after inoculation on susceptible and resistant wheat cultivars, non-host cereals and five species of dicotyledons. Field observations (Abiev et al., 1982) indicate, that wild grasses can be used as hosts by wheat yellow rust and represent a possible source of infection for wheat crops. Some work on the development of incompatible host-parasite combinations has been done using fluorescence techniques (Niks, 1981; 1982). A preliminary experiment was carried out to investigate the possibilities of growth experiments with cereal leaf rust fungi on non-host cereal cultivars. No replication was applied in this

Table 5.4
Radial growth (µm) of some leaf rust fungi on host and non-host cereal cultivars and on susceptible and resistant hosts.●

a) Barley rusts on three barley cultivars

Cultivar Midas	Day 2	Day 4	Day 7
Isolate *)			
BBR race A	20	46	120
BBR 83-2	23	68	238
BYR race 1	18	44	260
Cultivar CI 1242			
Isolate			
BBR race A	22	47	166
BBR 83-2	26	45	218
BYR race 1	22	43	55
Cultivar Berac			
Isolate			
BBR race A	25	38	158
BBR 83-2	27	91	224
BYR race 1	24 +)	42	399

b) Other relationships

Cultivar Armada (wheat)	25 hours	113 hours	209 hours
Isolate			
BBR 83-2	39	0	0
BYR race 1	24	0	0
OCR FR 1	9	12	0
RBR 70-1	27	0	0
WBR 77-22	25	141	508
Cultivar Berac (barley)			
Isolate			
BBR 83-2	22	80	413
BYR race 1	22	85	163 +)
OCR FR 1	37	75	285
RBR 70-1	24	15	0
WBR 77-22	19	0	0
Cultivar Maris Tabard (oats)			
Isolate			
BBR 83-2	25	0	0
BYR race 1	0	0	0
OCR FR 1	37	195	309 +)
RBR 70-1	22	0	0
WBR 77-22	17	0	102

Table 5.4 continued

Cultivar Rheidol (rye)

Isolate *)		25 hours	113 hours	209 hours
BBR	83-2	26	0	0
BYR	race 1	22	84	515
OCR	FR 1	36	21	1056
RBR	70-1	39	194	239 +)
WBR	77-22	21	0	0

0.5

● radial growth = 1/2(length of colony * breadth of colony)

*) BBR = barley brown rust (P. hordei)
BYR = barley yellow rust (P. striiformis)
OCR = oat crown rust (P. coronata)
RBR = rye brown rust (P. recondita)
WBR = wheat brown rust (P. triticina)

+) estimated values only

preliminary experiment. The results from the preliminary experiments are presented in Table 5.4. The present investigation aimed to study further the development of cereal rusts on non-host cereals.

Materials and Methods:

Two isolates of barley brown rust (P. hordei Otth) and one isolate each of oat crown rust (P. coronata Corda), rye brown rust (P. recondita Rob. & Desm.), wheat brown rust (P. triticina Eriksson) and wheat yellow rust (P. striiformis Westend.) were inoculated onto detached leaves of two cultivars of barley (cvs. Berac and CI 1243), oats (cvs. Maris Tabard and Bond), rye (cvs. Rheidol and Dominant) and wheat (cvs. Armada and Michigan Amber) and one cultivar of triticale (cv. Bush) respectively. The inoculation was carried out in a spore settling tower (Fig. 5.1). The leaves were kept on 80 ppm benzimidazole water agar (0.7%) in plastic petri dishes. For the inoculation freshly collected urediniospores were used and an inoculum density was chosen

which ensured the presence of at least 10 and not more than 60 spores / cm^2. After inoculation the petri dishes were transferred to controlled environment cabinets and kept at 14 °C at 16 h light and 8 h darkness. As the water agar provides sufficient humidity to keep the air inside the petri dishes saturated, no controlled humidity was required.

Sections of 10-15 mm length were cut off the leaves after 3, 7 and 11 days, fixed in boiling lactophenol : ethanol (1:2) for 90 seconds and stained for fluorescence microscopy in Calcofluor White M2R New, in the procedure after Rohringer et al. (1977; and see Appendix 2.2). The specimens were then examined with a Leitz Ortholux 2 microscope, fitted with epifluorescence equipment, and the length and breadth of the invading fungal mycelium were measured. On day 21, the length and breadth of colonies were measured on unstained leaves. This was possible only where sporulation or distinct chlorosis occurred and some latent infections thus escaped the assessment. For the qualitative assessment of sporulation, a four class system was adopted: with 0 = none, 1 = very little, 2 = reduced and 3 = full sporulation. The quantitative assessment was carried out using a suction method for spore collection (Helfer 1985) and measuring the spore concentration photometrically at 500 nm. The experiments were carried out using three replicates of each host-isolate combination. Where unexpected sporulation on non - hosts occurred, spore samples were collected and the identity of the isolate was tested on varieties of the normal host plant.

Results:

The urediniospores of all isolates germinated well on all cereal cultivars, forming appressoria over their stomata (not P. striiformis!) but some of the germ tubes of rusts which were specialised on glabrous

hosts (barley and oats) grew in a somewhat disorientated manner on the hirsute cultivars (rye, triticale and wheat), thus often missing the nearest stomata. The same could be observed with germ tubes of rusts normally developing on hirsute leaves, when they were inoculated onto glabrous cultivars. Substomatal vesicle (SSV) formation and the development of infection hyphae could be observed after appressorium formation. Only in very rare cases could an abortion of the infection be recorded at appressorium formation (once with barley brown rust on wheat) or SSV formation (wheat yellow rust on oats). In many combinations external formation of SSV without stomatal penetration could be observed: rye brown rust formed external SSV when inoculated on oats and triticale, wheat brown rust on barley and oats, and wheat yellow rust on all examined hosts. This phenomenon had first been noticed on barley yellow rust (P. striiformis) on barley and other cereals (Helfer, unpublished results, Kellock, personal communication). Early abortion sensu Niks (1982) occurred in most non-host combinations, but not necessarily at all penetration sites. The results 3 days after inoculation include these early abortions. After 7 days, the aborted fungal tissue did not take up the fluorescent stain any more, possibly because it was dead and decomposing. This explains the disappearance of positive growth values in incompatible reactions after three days.

Table 5.5 shows the radial growths of the six pathogens on nine cereal cultivars on days 3, 7, 11 and 21. These are represented as the geometric means of length and breadth. No statistically significant interaction could be detected between cultivar and rust isolate on day 3. On the later dates however a strong interaction occurred, indicating the susceptibility and resistance of cultivars to rusts. Several inconsistencies could be observed here: oat crown rust was observed

Table 5.5
Radial growth (µm) of leaf rust fungi on host and non-host cereal cultivars ▲

Cultivar Berac (barley) Isolate *)		Day 3	Day 7	Day 11	Day 21
BBR	race A	44	272	644	1250
BBR	84-1	61	259	633	1426
OCR	FR-1	87	73	239	0
RBR	70-1	21	9	0	0
WBR	80-21	18	5	209	0
WYR	108E137	26	73	679	0
Cultivar CI 1243 (barley)					
Isolate					
BBR	race A	52	98	260	556
BBR	84-1	74	343	736	1041
OCR	FR-1	61	0	0	0
RBR	70-1	25	0	0	0
WBR	80-21	22	48	290	135
WYR	108E137	14	0	0	0
Cultivar Maris Tabard (oats)					
Isolate					
BBR	race A	4	0	0	0
BBR	84-1	10	0	0	0
OCR	FR-1	68	476	768	2236
RBR	70-1	26	25	0	0
WBR	80-21	16	0	0	0
WYR	108E137	25	0	0	322
Cultivar Bond (oats)					
Isolate					
BBR	race A	15	0	0	0
BBR	84-1	11	0	0	0
OCR	FR-1	73	455	1482	2636
RBR	70-1	21	0	0	0
WBR	80-21	7	0	80	0
WYR	108E137	23	0	0	0
Cultivar Rheidol (rye)		Day 3	Day 7	Day 11	Day 21
Isolate					
BBR	race A	39	0	0	0
BBR	84-1	27	39	0	0
OCR	FR-1	84	206	458	0
RBR	70-1	57	572	1338	2383
WBR	80-21	38	218	569	676
WYR	108E137	19	0	1516	0

continued next page

Table 5.5 continued

Cultivar Dominant (rye)					
Isolate					
BBR	race A	28	16	0	0
BBR	84-1	37	11	0	0
OCR	FR-1	158	101	617	0
RBR	70-1	59	542	1478	2883
WBR	80-21	26	167	161	887
WYR	108E137	11	13	0	0
Cultivar Bush (triticale)					
Isolate					
BBR	race A	30	16	0	0
BBR	84-1	32	26	0	0
OCR	FR-1	101	0	0	0
RBR	70-1	43	0	247	0
WBR	80-21	30	347	652	2433
WYR	108E137	19	92	0	0
Cultivar Armada (wheat)					
Isolate					
BBR	race A	26	0	0	0
BBR	84-1	31	0	0	0
OCR	FR-1	3	0	0	0
RBR	70-1	13	0	0	0
WBR	80-21	71	424	853	1739
WYR	108E137	32	568	1344	1935
Cultivar Michigan Amber (wheat)					
Isolate					
BBR	race A	28	19	0	0
BBR	84-1	21	10	0	0
OCR	FR-1	30	532	1	0
RBR	70-1	28	0	0	0
WBR	80-21	69	393	967	1741
WYR	108E137	10	153	2960	4268
SED		± 26.62	± 100.64	± 393.4	± 614.8
**		± 24.67	± 97.73	± 317.5	± 604.3

*) BBR = barley brown rust (P. hordei)
OCR = oat crown rust (P. coronata)
RBE = rye brown rust (P. recondita)
WBR = wheat brown rust (P. triticina)
WYR = wheat yellow rust (P. striiformis)

** for same levels of rust

0.5

▲ radial growth = 1/2(length of colony * breadth of colony)

Table 5.6

Sporulation of cereal leaf rusts on nine host and non-host cereal cultivars.

(a) Qualitative assessment (Scale 0-3)

Isolate *) **)		BBR	BBR	OCR	RBR	WBR	WYR
Cultivar *)		race A	84-1				
Berac	(B)	2.3	2.7	0.0	0.0	0.0	0.0
CI 1243	(B)	0.7	2.3	0.0	0.0	0.3	0.0
Maris Tabard	(O)	0.0	0.0	3.0	0.0	0.0	1.0
Bond	(O)	0.0	0.0	3.0	0.0	0.0	0.0
Rheidol	(R)	0.0	0.0	0.0	2.7	1.0	0.0
Dominant	(R)	0.0	0.0	0.3	2.5	1.0	0.0
Bush	(T)	0.0	0.0	0.0	0.0	2.0	0.0
Armada	(W)	0.0	0.0	0.0	0.0	2.3	1.7
Michigan Amb.	(W)	0.0	0.0	0.0	0.0	3.0	2.0

SED ±0.4127

0 = no sporulation
1 = little sporulation
2 = reduced sporulation
3 = abundant sporulation
values represent means of three replicates

(b) Spore number production of single infections measured by light absorbance at 500 nm (X 10^3 spores).

Isolate		BBR	BBR	OCR	RBR	WBR	WYR
Cultivar		race A	84-1				
Berac	(B)	33	34	0	0	0	0
CI 1243	(B)	<30	33	0	0	32	0
Maris Tabard	(O)	0	0	46	0	0	0 +)
Bond	(O)	0	0	45	0	0	0
Rheidol	(R)	0	0	0	37	<30	0
Dominant	(R)	0	0	0	34	<30	0
Bush	(T)	0	0	0	0	35	0
Armada	(W)	0	0	0	0	37	48
Michigan Amb.	(W)	0	0	0	0	41	207

SED ±40

+) = sporulation in one case but not in sample

*) B = barley
O = oats
R = rye
T = triticale
W = wheat

**) BR = brown rust
CR = crown rust
YR = yellow rust

growing quite substantially (580 µm) on Michigan Amber wheat on day 7 but not on any of the other dates; wheat yellow rust developed well in the barley cultivar Berac (679 µm on day 11) and in the rye cultivar Rheidol (1484 µm on day 11) without producing visible infection on day 21; the same was true to a lesser extent for oat crown rust on barley cultivar Berac (239 µm) and the two rye cultivars (458 and 652 µm respectively); wheat yellow rust on the oat cultivar Maris Tabard could be observed only on one date (day 21) when it sporulated.

On day 7 sporulation began to occur in the wheat / wheat brown rust combination, with the other combinations following later. On day 21 all compatible combinations were sporulating, including some unexpected ones: P. triticina formed spores on the barley cultivar CI 1243, the two rye cultivars and triticale, and P. striiformis sporulated abundantly on Maris Tabard oats in one replicate. Table 5.6 shows the mean values of sporulation and the spore production measured by light absorbance in a colorimeter at 500 nm. Wheat yellow rust produced the highest response from single infection sites. The sporulation of the incompatible barley brown rust isolate race A on barley cultivar CI 1243 was very low, as were sporulations from other incompatible combination. A hypersensitive response was evident in the barley brown rust race A / CI 1243 combination.

Discussion:

Gaeumann (1959) mentions many possible hosts for the five rust species investigated in this paper. For P. hordei he states 12 host species, all of which belong to the genus Hordeum. P. coronata is described in three groups and 16 formae speciales on 256 host species comprising barley, oats, rye and wheat (all attacked by the forma

specialis avenae Eriksson). For P. triticina, 29 host species, including rye and barley, are descibed. The host range of P. recondita (P. dispersa), according to Gaeumann, consists of 12 species, none of them being cereals apart from rye. Puccinia striiformis (P. glumarum) appears in five different formae speciales on 102 host species, but physiologic diversity has been reported between different acquisitions of the same physiologic form.

Wilson and Henderson (1966) describe three host species for P. hordei, 25 for P. coronata, two for P. recondita (f.sp. recondita), three for P. triticina (P. recondita f.sp. tritici) and more than 10 host species for P. striiformis, but not including oats. In field studies carried out in the USSR, Abiev et al. (1982) observed infection on seven wild grass species with wheat yellow rust but reported that oats and barley grown in the region were free of symptoms; reinfection of wheat was possible with urediniospores from three wild grasses. Ogle and Brown (1971) showed that wheat stem rust formed normal appressoria and SSV on non-host cereals, but failed to produce penetration structures on the five species of dicotyledons which had been included in their experiment. They discovered no significant differences in the colony area until 72 hours post inoculation and their observations on the pattern of development of wheat stem rust on compatible and incompatible hosts was similar to that found with wheat brown rust tested on the cereal species in this experiment. Niks (1981) showed that barley brown rust readily formed appressoria on wheat but failed to penetrate lettuce. Clifford and Roderick (1978) showed that, in incompatible barley / P. hordei interactions, some colonies still reached considerable size whilst the distribution of colony sizes differed significantly.

Although the system used in this experiment is highly artificial, it has been shown that resistance responses in detached leaves were identical or similar as on whole plants provided benzimidazole was added to the supporting medium (Samborski et al., 1958; Bjoerkmann, 1960). In both the preliminary studies, described in the introduction, and in the main experiments, sustained infection by particular rust species were usually confined to their respective appropriate hosts. However, in some sporadic cases a rust species developed infection and produced spores on a non - host cereal. Every care was taken to ensure that no cross contamination occurred in these studies and further work to investigate this phenomenon is recommended.

CHAPTER 6

General conclusions

In the present studies features of colony growth and development of the leaf rust fungi of temperate cereals have been researched. Taxonomical aspects, culture characteristics, cytogenetics, and growth and development responses of these pathogens in hosts and non - hosts have also been considered.

In attempting to clarify the taxonomical position of the cereal leaf rusts, classical taxonomical features as well as developmental characteristics of the urediniospore stage were taken into account. Most questions concerning the taxonomy of these fungi are now resolved in the literature and, from urediniospore stage features along with the partial use of other developmental stages, clear and unmistakable distinction of the yellow rusts from the brown rusts and crown rust, as well as barley brown rust from the brown rusts of wheat and rye, can be made. On the other hand, the position with wheat and rye brown rust has been less well defined. The present studies, where special attention was paid to differences which were independent of the plants the parasites were growing on, showed clear morphological as well as physiological differences between wheat and rye brown rust, and provide evidence in keeping with the view that these two organisms should be regarded as two distinct species.

Studies on the ability of rusts to grow in different culture

conditions included experiments to induce the axenic culture of some isolates of cereal leaf rusts. These experiments, however, did not lead to any successful saprophytic growth on complex culture media. The culture of leaf rusts on detached seedling leaves of their respective host plants, on the other hand, was successful with all isolates. The most satisfactory detached leaf culture was achieved on 0.7 % water agar containing 80 ppm benzimidazole, and at 14 °C in a 16 hour light / 8 hour darkness regime. The seedling leaves of barley, oats, rye and wheat could be maintained in good condition for 4 to 5 weeks, which allowed enough time for their rusts to show a substantial development in compatible host - rust relationships.

Although the somatic recombination of virulence genes in yellow rust of wheat has been reported in previous work, no recombination events could be recorded in the present studies. When the nuclear condition of germinating spores as well as fusion events prior to host penetration were investigated, the nuclear condition of the isolates used was always dikaryotic and no fusion phenomena between germ tubes, appressoria or substomatal vesicles could be recorded. However, somatic recombination of virulence genes in the rusts is apparently a rare phenomenon and may also be dependant on the genetic background of the isolates involved. As it leads to more complex virulence combinations, more research into its frequency and mechanism is recommended.

From the results of work on growth and development patterns of the cereal leaf rusts, it may be concluded that the efficiency of spore germination is more variable in yellow rust isolates than in other cereal leaf rusts. Also germ tube penetration of brown rusts and crown rust isolates on their respective hosts, involving appres-

sorium formation, was more effective than the direct penetration evidenced in yellow rust isolates. However, once host penetration had successfully taken place, colony growth and development of yellow rust isolates were more extensive than that of brown rusts and the number of spores produced from successful yellow rust infections, with large numbers of sori, exceeded by far the spore production in the other leaf rust pathogens where restricted uredi-niosori numbers per colony were recorded. In this way the initial disadvantage in germination and host penetration in the yellow rusts was offset by more extensive colonisation and spore production. This difference in growth and development characteristics between the different rusts reflects their different adaptations to sustaining high rates of infection during their epidemic development.

Colony growth patterns in host and non - host leaves usually diverged at an early stage of rust development. In some cases, however, considerable colony development in non - host plants was observed. Further study of this phenomenon in developing a deeper understanding of host - pathogen interactions is suggested.

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APPENDIX 1.1

List of cereal varieties used in the experiments:

Barley: Hordeum vulgare

Cultivar	Resistance genes *)
Astrix	BR 0 (?); YR 1
Berac	BR 0 +); YR 0 +)
Bigo	BR 6; YR 2
Bolivia	BR 6; YR ?
Cebada Capa	BR 7; YR ?
CI 1243	BR 9; YR ?
Egypt 4	BR 8; YR ?
Gold	BR 4; YR ?
Keg	BR 0; YR 0
Mazurka	BR ?; YR 1,2(?)
Midas	BR 0 +); YR 0
Peruvian	BR 2; YR (?)
Quinn	BR 5; YR (?)
Ribari	BR 3; YR 0
Simon	BR 0; YR 0
Sudan	BR 1; YR (?)
Varunda	BR (?); YR 2

Oats: Avena sativa

Cultivar	Resistance genes *)
Anthony	CR 1
Appler	CR 3
Bond	CR 4
Bondvic	CR 9
Landhafer	CR 5
Maris Tabard	CR 0 +)
Saia	CR 10
Santa Fe	CR 6
Trispernia	CR 8
Ukraine	CR 7
Victoria	CR 2

Rye: Secale cereale

Cultivar	Resistance genes *)
Dominion	BR 0 +)
Rheidol	BR 0 +)

Triticale:

Cultivar	Resistance genes *)
Bush	?

APPENDIX 1.1 continued

Wheat: Triticum aestivum

Cultivar	Resistance genes *)
Armada	BR 0 +); YR 12
Carstens V	BR (?); YR ?
Chinese 166	BR ?; YR 1
Clement	BR 1; YR 9
Compair	BR ?; YR 8
Heines VII	BR ?; YR 2
Heines Kolben	BR ?; YR 6
Heines Peko	BR ?; YR 6,1(?)
Hustler	BR ?; YR 1,2,13
Hybrid 46	BR ?; YR 3b,4b
Lee 1	BR 0; YR 7
Longbow	BR 5; YR 1,2,6,13
Mardler	BR 5; YR 1,2
Maris Bilbo	BR 2,9; YR 14
Maris Fundin	BR 2; YR ?
Maris Huntsman	BR 5; YR 2,13
Maris Ranger	BR 8(?); YR 6
Michigan Amber	BR 0; YR 0 +)
Moro	BR ?; YR 10
Nord Desprez	BR ?; YR ?
Norman	BR 2; YR 2,6
Rapier	BR ?; YR 2,4,(14)
Reichsberg 42	BR ?; YR ?
Riebesel 47/51	BR ?; YR 9
Sappo	BR 3; YR 0 +)
Spalding's Prolifique	BR ?; YR ?
Sportsman	BR 9; YR ?
Strubes Dickkopf	BR ?; YR ?
Suwon x Omar	BR ?; YR 3(?)
Vilmorin 23	BR ?; YR 3a,4a
Triticum spelta	BR ?; YR 5

*) BR = brown rust resistance
CR = crown rust resistance
YR = yellow rust resistance

+) Universally susceptible cultivars.

APPENDIX 1.2

List of rust isolates used in the experiments, their virulence and origin:

Oat crown rust *P. coronata*

Isolate	Virulence *)	Origin +)
Field isolate	OCV 1,2,4,5	ESA

Barley brown rust *P. hordei*

Isolate	Virulence *)	Origin +)
Race A	BBV 1,4,10	WPBS
76-12	BBV 1,2,3,4,6,8,9,10	WPBS
83-1	BBV 1,2,4,5,6,8,9,10	ESA
83-2	BBV 1,2,4,5,6,8,10	ESA

Rye brown rust *P. recondita*

Isolate	Virulence *)	Origin +)
70-1	RBV ?	WPBS

Barley yellow rust *P. striiformis*

Isolate	Virulence *)	Origin +)
Race 1	BYV 1	ESA

Wheat yellow rust *P. striiformis*

Isolate	Virulence *)	Origin +)
Race 37E132	WYV 1,2,6	PBI
Race 41E136	WYV 1,2,3	PBI
Race 104E137	WYV 2,3,4	PBI
Race 104E137 W	WYV 2,3,4	PBI
Race 108E9	WYV 3,4,6	PBI
P 631	WYV 1,2,3,4,6	NIAB
P 71-493	WYV 1,2,3,7	NIAB
P 72-23	WYV 2,3,4	NIAB
P 75-27	WYV 2,3,4,14	NIAB
P 76-15	WYV 2,3,4,8,(9)	NIAB
P 75-109	WYV 2,3,4,6	NIAB
P 80-21	WYV 2,4,(6)	NIAB
P 81-11	WYV 1,2,3	NIAB

APPENDIX 1.2 continued

Wheat brown rust *P. triticina* Isolate	Virulence *)	Origin +)
WBRS 74-2	WBV 5,8,9	WPBS
WBRS 77-22	WBV (1),5,8,9	WPBS
WBRS 79-4	WBV 2,5,8,9	WPBS
WBRS 79-21	WBV 2,3,5,8,9	WPBS
ESA 83-1	WBV 1,2,5,8,9	ESA
ESA 83-2	WBV 1,2,3,5,8,(9)	ESA

*) OCV = oat crown rust virulence
BBV = barley brown rust virulence
RBV = rye brown rust virulence
BYV = barley yellow rust virulence
WYV = wheat yellow rust virulence
WBV = wheat brown rust virulence

+) ESA = Edinburgh School of Agriculture
NIAB = National Institute for Agricultural Botany
PBI = Plant Breeding Institute, Cambridge
WPBS = Welsh Plant Breeding Station

APPENDIX 2.1

Methods for light microscopy (LM):

i) Stereo microscopy:

- Specimens were observed directly, without preparation using a Kyowa stereo microscope fitted with glass fiber incident light and dark field light sources.

ii) Transmission microscopy:

- Specimens on agar were observed without preparation, covering them with a microscope cover slip.
- Spores for measurements were suspended in immersion oil prior to observation.

APPENDIX 2.2

Preparation technique for fluorescence microscopy (Calcofluor; after Rohringer et al., 1977):

a) Collection of samples:
- cut leaves to approx. 5 mm X 20 mm on agar using scalpel
- transfer leaf sections into glass vials containing lactophenol / ethanol (1:2)

b) Fixation and clearing:
- boil for 90 s in lactophenol / ethanol (at about 81 °C in the water bath)
- leave in the fixative for 16 h (overnight).
- wash in 50 % v/v ethanol 2 X 15 min.
- wash in 0.05 N NaOH 2 X 15 min.
- wash in dist. water 3 X 10 min.
- transfer to 0.1 M Tris / HCl buffer pH 8.5 and leave for 30 min.

c) Staining:
- transfer to Calcofluor White M2R New (Cynamide) 0.1 % in 0.1 M Tris / HCl buffer pH 8.5 for 5 min.
- wash in dist. water 4 X 10 min.
- transfer to glycerol 25 % for 30 min.

d) Mounting:
- mount in glycerol 50 % with 1 ml/l lactophenol for preservation

e) Observation:
Slides were examined using a Leitz Ortholux II microscope fitted with epifluorescence equipment using a Wotan HBO 100 W/2 bulb and excitation filters at 430 and 515 nm and barrier filters at 460 and 490 nm.

APPENDIX 2.3

Methods for scanning electron microscopy (SEM):

i) Freeze drying

a) Collection of samples:
- cool leaves to 3-4 °C
- cut leaves to approx. 5 X 5 mm using scalpel
- immerse sections in liquid nitrogen
- after freezing transfer to glass vials placed in liquid nitrogen on a metal plate (brass approx. 5 mm thickness), insulated by polystyrene.

b) Drying:
- transfer of polystyrene beaker to vacuum chamber
- application of vacuum at 0.05 torr for 10 to 16 h
- storage of the dried specimen in glass vials over blue silica gel

c) Mounting & sputter coating:
- mounting of specimens onto SEM specimen stubs using double sided adhesive tape and coloid silver to provide enhanced contact
- coating of specimens with gold for 3 min. in a polaron sputter coater at 30 mAmp. in an argon atmosphere.

d) Observation:
- observation in a Jeol SEM at an acceleration voltage of 10 kV.

ii) Critical point drying

a) Collection of samples:
- cut leaves to approx. 5 X 5 mm using scalpel
- prefixation in 2.5 % glutaraldehyde in 0.006 M phosphate buffer pH 7.2 for 2 h to a few days
- rinse in buffer for 6 X 10 min.

b) Fixation & drying:
- fixation in osmium tetroxide in buffer containing 50 mM $MgCl_2$ for 2 h
- rinse in water for 6 X 10 min
- dehydrate in ethanol series at 30, 50, 70, 85, 95 and 100 %, 10 min in each step
- transfer to critical point dryer in ethanol
- critical point drying in Balzers Critical point drying apparatus using liquid CO_2
- storage of the dried specimens in glass vials over blue silica gel.

c) & d) as for freeze drying.

APPENDIX 3.1

Medium for the axenic culture of rusts
(Bushnell, 1968).

- Evans' Peptone 0.1 %
- glucose 3 %
- Czapek's mineral salts
- agar 2 %
- adjust pH to 6.4 using HCl
- autoclave at 121 °C for 30 min.

APPENDIX 3.2

Procedure used for the Giemsa stain:

- drying of spores onto the microscope slide covered with agar
- fixation in ethanol / acetic acid (3:1) for 12 h
- rinse in 70 % ethanol 2 X 10 min
- rinse in water 2 X 10 min
- hydrolysis in 1 N HCl at 60 °C for 7 min
- rinse in 0.06 M phosphate buffer pH 7.0 for 3 X 10 min
- transfer to Giemsa stain 10 % in the same buffer for 1 to 4 h
- rinse in buffer 3 X 10 min
- dry specimens and mount in Entellan

APPENDIX 4.1

Preparation technique for fluorescence microscopy (Mithramycin; modified after Anikster, 1983):

a) Collection of samples:
- cut leaves to approx. 5 mm X 20 mm on agar using scalpel
- transfer leaf sections into glass vials containing lactophenol / ethanol (1:2)
- prefixation in 5 % glutaraldehyde in 0.06 M phosphate buffer pH 7.0 for 30 min.

b) Fixation and clearing:
- boil for 90 s in lactophenol / ethanol (at about 81 °C in the water bath)
- leave in the fixative for 16 h (overnight).
- wash in 50 % v/v ethanol 2 X 15 min.
- wash in 0.05 N NaOH 2 X 15 min.
- wash in dist. water 3 X 10 min.
- transfer to 0.1 M Tris / HCl buffer pH 8.5 and leave for 30 min.

c) Staining:
- transfer to Calcofluor White M2R New (Cynamide) 0.1 % in 0.1 M Tris / HCl buffer pH 8.5 for 5 min.
- wash in dist. water 4 X 10 min.
- transfer into 100 µg/ml mithramycin in 0.06 M phosphate buffer with 50 mM $MgCl_2$ for 10 min.
- rinse in buffer 2 X 10 min.
- transfer to glycerol 25 % for 30 min.

d) Mounting:
- mount in glycerol 50 % with 1 ml/l lactophenol for preservation

e) Observation:
Slides were examined using a Leitz Ortholux II microscope fitted with epifluorescence equipment using a Wotan HBO 100 W/2 bulb and excitation filters at 430 and 515 nm and barrier filters at 460 and 490 nm.

APPENDIX 5.1

Note in the Bulletin of the British Mycological Society 19,68-69.

A simple method of collecting spores of fungal leaf pathogens

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For many quantitative experiments on air-borne diseases the assessment of the spore production of a pathogen on a host plays an important role. The spore number is a more sensitive and meaningful measure of spore production than spore weight and, particularly with low spore numbers, weighing may give substantial errors or be impractical. Suction methods to collect spores have been used by a number of researchers (Tervet et al., 1951, Errington and Powell, 1969, Browder, 1971, Bartlett and Bainbridge, 1978). Many of the described devices are not suitable for quantitative assessment when there are few spores or are impractical for large numbers of measurements. This note describes a simple method of assessment of spore numbers. It has been used with spores of Puccinia and Erysiphe species, giving consistant results.

A 1000 µl plastic pipette tip which has been slightly bent by heat is coated on the inside with a mineral or vegetable oil of medium viscosity. The pipette tip is then connected to a vacuum pump set at a low suction pressure (-25 mm Hg) and the spores are drawn in, moving the tip over the leaf with the inlet 1-2 mm from the leaf surface. After the collection the pipette tip is rinsed with a defined amount of oil (200-100 µl), the spores are evenly suspended and suspension samples are counted in a haemocytometer. The method is particularly useful for detached leaves and small trials, where one leaf represents one replicate. Up to 100 assessments can easily be done within one day.

At a suction pressure of -25 mm Hg the air speed at the inlet of the pipette is around 56 m/s, at the outlet around 0.55 m/s. Control experiments showed that at this setting only few spores (5%) escaped into a filter which was attached to the outlet of the pipette.

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APPENDIX 5.2

Abstract of a paper given at the VI th European and Mediterranean Cereal Rusts Conference in Grignon (France), 4-7 September 1984.

Infection responses to different levels of inoculum density in Puccinia hordei and Puccinia striiformis on barley.

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Urediniospore suspensions of Puccinia hordei and Puccinia striiformis, made up at various concentrations ranging from 1 to 1140 spores / cm^2, were applied to both plants and detached leaf segments of fully susceptible cultivars of Hordeum vulgare. After spray inoculation the plants or leaves were kept in a controlled environment and regularly screened for the progress of infection. The rate of penetration was assessed by scanning electron microscopy and, from the onset of sporulation, spore production was monitored.

Preliminary experiments with single spore inoculum showed that the two rust species exhibited different patterns of colonisation: P. striiformis gave many initial sori from every successful penetration while P. hordei produced only one initial sorus. In the case of P. hordei germination rate and rate of penetration of host tissues were influenced by the inoculum density. Germination rates increased with density up to 100 spores / cm^2 and then declined slightly : penetration rate increased with increasing density up to 250 spores / cm^2. The actual spore production was highest at an inoculum density of around 250 spores / cm^2. Comparable studies are in progress with P. striiformis.